

DENITRIFICATION OF HIGH STRENGTH LANDFILL LEACHATE USING GARDEN REFUSE COMPOST AND PINE BARK.

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**Submitted in fulfilment of the requirements for a research Masters of
Science in Engineering in the Civil Engineering Programme, University
of KwaZulu-Natal, Durban, South Africa.**

DECEMBER 2009

As the candidate's Supervisor I agree/do not agree to the submission of this dissertation.

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DECLARATION

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PREFACE

The research presented in this dissertation was carried out under the supervision of Prof. Cristina Trois of the School of Civil Engineering, Surveying and Construction, University of KwaZulu Natal, Durban, South Africa. This dissertation has been compiled in accordance with *The Style Guide for Dissertations*, prepared by the Faculty of Engineering of the University of KwaZulu Natal, Durban and represents work written by Björn Plüg, unless otherwise stated in the text.

ACKNOWLEDGEMENTS

I would like acknowledge my supervisor, Prof. Cristina Trois, for her tireless professional guidance and support during the period of this masters research dissertation.

I would especially like to thank Sheila Arnold for her collaboration on this project and Gloria of the UKZN Civil Engineering Environmental Laboratory for her constant assistance.

To the Technicians and Technical Staff of the UKZN Civil Engineering Workshop thank you for your contribution towards the construction of the column experiments and preparation of the Reactor Room.

I would like to thank Durban Solid Waste for providing me with this valuable opportunity to research a significant topic in relation to Environmental Engineering.

Three Undergraduate students assisted with the Laboratory work as part of their Honours degree.

ABSTRACT

Landfill leachate, a toxic by-product formed through the decomposition of organic matter, is harmful to the environment and human health. After nitrification, the concentration of nitrate in discharged leachate may still present a potential threat to the environment. Further denitrification is required to reduce the high concentrations of nitrates in the nitrified effluents to below discharge limits. The eThekweni Municipality is currently nitrifying leachate from the Mariannhill Landfill site in a Sequencing Batch Reactor plant. After closure of the landfill (expected in 2012) the effluents from the plant will not comply with discharge limits, requiring an ad-hoc treatment. Denitrification, the conversion of nitrates to nitrogen gas, occurs in the presence of a carbon source in an anaerobic environment. Expensive methods are currently employed worldwide; however these tend not to be a viable solution for developing countries.

This investigation aims at identifying an efficient, cost effective, feasible alternative to expensive easily biodegradable carbonaceous materials such as methanol, which promotes the use of natural organic sources such as pine bark and garden refuse. These organic substrates contain relatively high amounts of carbon and are readily available in the major eThekweni landfills.

The suitability of these substrates as carbon sources for denitrification, were assessed using characterisation tests, small scale batch tests and larger scale columns. The preliminary stage of the research was to comprehensively characterise the substrates through conventional testing done on both the solid substrates and their eluates. The batch tests were conducted at 3 nitrate concentration levels: 100, 500 and 2000 mg/ℓ. A synthetic nitrate solution was used to simulate the treated Mariannhill Landfill site leachate. Substrates selected for large scale experiments in columns were, the fresh pine bark, the fresh Commercial Garden Refuse (CGR) and immature Commercial Garden Refuse (CGR) compost. Two nitrate concentrations (500 and 2000 mg/ℓ) at two different flow rates were used for the column campaign. Finally durability tests were conducted on previously used substrates of pine bark and immature compost to determine the period for which the substrates could be used as a means for denitrification before replacement was necessary.

The characterisation tests indicated that the fresh materials had higher carbon to nitrogen ratios than the composted substrates. The CGR RAW substrate had the highest carbon to nitrogen ratio of 90.19 and although the pH value of 5.45 falls just outside the optimum range for denitrification of 6 – 8, it was expected that this would be the best performing substrate.

All the batch tests showed positive results, with regard to achieving full denitrification with a 100% removal occurring in 5 of the 6 substrates, at all the different nitrate concentrations. The only substrate not to achieve full denitrification was the pine bark. The best performing substrate was the CGR RAW which achieved full denitrification at the highest nitrate concentration of 2000 mg/l between 9 – 12 days.

The column tests reflected promising results at $C_o = 500$ mg/l during experiment 1, with all 3 achieving full denitrification. Once again the CGR RAW substrate columns reflected the best results. The column at 500 mg/l displayed a HRT of 8.06 days was required whereas the higher concentration of 2000 mg/l required a HRT of 8.40 days. During experiment 2, the CGR RAW substrate column at 500 mg/l was the only one to achieve 100% nitrate removal. A HRT time required for full denitrification is less than 3.54 days.

Further studies need to be done at different flow rates and concentrations to ensure that the reactor is robust and flexible to deal with the change in quality of the leachates during the life of the landfill. Lower concentrations need to be investigated to determine whether the substrates are suitable for all ranges of nitrates and leachates. The use of a combination of substrates as well as different levels of maturity is also required to determine the ideal material for their implementation in a full-scale reactor in the future. Larger scale reactors and different reactor configurations need to be investigated.

GLOSSARY

Ammonia	NH ₃
Biochemical Oxygen Demand	BOD ₅
Carbon to nitrogen ratio	C/N
Chemical Oxygen Demand	COD
Commercial Garden Refuse	CGR
Commercial Garden Refuse 10 Weeks	CGR 10
Dome Aeration Technology	DAT
Domestic Garden Refuse	DGR
Domestic Garden Refuse 10 Weeks	DGR 10
Fresh Commercial Garden Refuse	CGR RAW
Liquid to solid ratio	L/S
Moisture content	w
Nitrates	NO ₃
Nitrites	NO ₂
Pine Bark	PB
Respiration Index	RI ₇
Sequencing Batch Reactor	SBR
Total solids	TS
Turned Windrow	TW
Volatile solids	VS

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CHAPTER 1

1. INTRODUCTION

Landfill leachate, which is a toxic by-product formed through the decomposition of organic matter, is harmful to both the environment and human health. After nitrification, the concentration of nitrates in the discharged leachate may still present a potential threat to the environment. Further denitrification is often required to reduce the high concentrations of nitrates in the nitrified effluents to below the discharge limits. The eThekweni Municipality is currently nitrifying leachate from the Mariannhill Landfill site in a Sequencing Batch Reactor plant. The treated effluent is then used as dust suppressant. The typical ranges of nitrate concentrations (Nitrate + Nitrite mg NO_3/ℓ) displayed by the treated landfill leachate produced by the Sequencing Batch Reactor (SBR) at the Mariannhill Landfill site are between 8 – 2120 mg NO_3/ℓ . After closure of the landfill (expected in 2012) the effluents from the plant will not comply with the discharge limits of wastewater into a water resource, as enforced by DWAF with a General Limit of 15 mg NO_3/ℓ and a Special Limit of 1.5 mg NO_3/ℓ (DWAF - General Authorisations in terms of Section 39 of the National Water Act, 1998). Thus an ad-hoc treatment will be required.

Biological denitrification, the conversion of nitrates to nitrogen gas, is facilitated by microbes. The micro-organisms capable of reducing nitrates require the presence of an external carbon source as an electron donor, usually in an anaerobic environment (Ovez et al., 2006). Expensive easily biodegradable carbonaceous materials are currently employed around the world; however these methods tend not to be a viable solution for developing countries and are not suited for large scale, field applications (Tsui et al., 2007; Volokita et al., 1995)

This investigation aims at identifying an efficient, cost effective and feasible alternative to expensive easily biodegradable carbonaceous materials, that promotes the use of natural organic resources such as pine bark and raw and composted garden refuse and that are suitable for large scale, field application. These organic substrates contain relatively high amounts of carbon and are readily available in the major eThekweni landfills.

The main objectives of this dissertation are:

- To determine the efficiency and performance of using a variety of organic substrates as carbon sources for the nitrate removal from treated landfill leachate.
- To assess their kinetics and efficiency of nitrate removal in different environmental conditions and flow rates.
- To investigate and compare the loading rates as well as hydraulic retention time of three of the substrates in a fixed bed reactor, simulated using leaching columns.
- To examine the durability and denitrification capabilities of these carbon sources over an extended period of time.

The investigation of the efficiency, performance and feasibility of nitrate removal using a variety of 6 substrates in the denitrification process was determined by means of laboratory testing. The selection of substrates was based on their suitability as natural organic carbon sources, their availability and aptness for large scale, field application. Thus pine bark and raw and composted garden refuse were chosen as these organic substrates contain relatively high amounts of carbon and are readily available in the major eThekweni landfills.

The suitability of the above substrates as carbon sources for denitrification was assessed using small scale dynamic batch tests (Tsui et al., 2007). The most suitable substrates were then selected for the large scale experiments in columns designed to simulate fixed bed reactors. These two methods of testing were chosen due to the particle size of the substrates and to simulate that of a fixed bed reactor, which is an attached biomass form of treatment. Synthetic nitrate solution was used to simulate the leachates produced from Mariannhill Landfill site.

The batch tests were conducted at 3 nitrate concentration levels: 100, 500 and 2000 mg/ℓ, according to the range of nitrate levels observed from the treated landfill leachate at Mariannhill Landfill site of 8 – 2120 mg NO₃/ℓ, to ascertain the loading capabilities of the 6 substrates in optimum conditions for denitrification with the maximum contact between the substrate and solution. The optimum conditions considered were those of a carbon to nitrogen ratio above 16, a pH between 6 and 8 as well as a temperature around 25°C - 30°C (Tsui et al., 2007).

Two nitrate concentrations (500 and 2000 mg/ℓ) and two different flow rates were used for the column campaign, with each combination being performed for over 4 weeks.

The leaching column studies were set up to accurately simulate fixed bed reactors (Tsui et al., 2007; Diaz et al., 2003; Volokita, 1995). It has been established that flow through a reactor improves the efficiency of denitrification on the postulation that water circulation favoured organic matter release and dispersion (Tsui et al., 2007; Diaz et al., 2003; Volokita et al., 1995).

The eluates from the columns were tested every day for nitrate concentrations, pH and temperature. Once a week ammonia (NH₃) and COD tests were also conducted. These tests were chosen to evaluate the rate of denitrification, the optimum conditions of temperature and pH as well as the release of both the carbon and nitrogen of the substrates.

Durability tests were conducted on previously used substrates of pine bark and immature compost to determine how long the substrate could be used as a means for denitrification before replacement is necessary.

In parallel to the above research, microbial analysis was conducted by De Combret (2009) to monitor the microbial diversity, growth, activity and patterns occurring during the denitrification process.

Background research on the topic was done making use of a wide range of available literature. The effectiveness of nitrate removal, using the different substrates as a carbon source, was determined through careful analysis and comparison of the experimental results with other studies (Tsui et al., 2007; Diaz et al., 2003; Volokita et al., 1995).

The outcomes of the experiments were to select an appropriate substrate based on percentage kinetics, rate of nitrate removal, to inform the future design of a full scale treatment system.

This dissertation comprises of several chapters. The literature review chapter is a collection of all the background research that has been conducted so as to fully understand the issues related to the research. The materials and methods used in the research dissertation are described in Chapter 3. All the results obtained in the research are presented and discussed in Chapter 4. The dissertation concludes with recommendations being made in Chapter 5. All the raw data examined in previous chapters have been provided in appendices A to D.

CHAPTER 2

2. LITERATURE REVIEW

2.1 Landfill Processes

Introduction

As the research conducted in this dissertation is applicable to the removal of nitrates from treated landfill leachate, the relevant processes of waste generation and disposal are thus reviewed so as to ascertain the significance of the research and its application to a landfill.

Waste Disposal Management

The volumes of waste being generated throughout the world are increasing every day. This waste poses a severe threat to the environment; due to the hazardous gaseous emissions and liquids produced. An integrated waste management system is the preferred method for waste disposal management. The waste management involves firstly waste generation. “Waste is generated from those activities in which the materials are identified as no longer being of value and are either thrown away or gathered for disposal” (Tchobanoglous et al., 1985). The waste is then collected, transported, processed and disposed of. This waste management system is shown in Figure 2.1. Landfilling is seen as the most viable and cost effective method of solid waste disposal. “Landfilling involves the controlled disposal of solid wastes on or in the upper layer of the earth’s mantle” (Tchobanoglous et al., 1985).

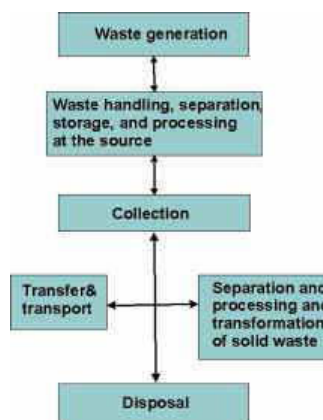


Figure 2.1: Integrated waste management system
(Tchobanoglous et al. 1993 as accessed from <http://www.brocku.ca/epi/ciet/whatis.htm> on 15/8/2009)

2.2. Mariannhill Landfill Site

The purpose of the research was aimed at determining the suitability of various organic carbon sources as a means to denitrify high strength leachate. In the future, these substrates are to be implemented in a full scale reactor at Mariannhill Landfill site. Thus the process in this specific site will be investigated. This landfill utilises a new generation design that successfully combines engineering issues, environmental concerns and conservation which need to be adhered to.

The Mariannhill Landfill site currently receives approximately 550 to 700 tons of solid waste per day. The solid waste is collected from the surrounding areas. Trucks and vehicles transporting the load of solid waste are weighed at the weigh bridge at the entrance of the site on arrival and once again at departure. The weight of the waste entering the site is thus recorded and the data captured. Thus the amount of waste entering the site can be monitored daily. The waste is then separated and sorted before being transported to the specific landfill cell. The loads of waste are then deposited at the cell and compacted. Once suitably compacted the waste is then covered using either a temporary blanket or a soil capping layer which would stimulate vegetation growth.

As a landfill is considered to be a large reactor where natural biodegradation processes take place within the waste bodies, landfill leachate and biogas are produced due to water passing through the waste body. Thus the protection of the environment from these potentially harmful landfill emissions needed to be addressed. At this site the use of a barrier system was utilised.

“Two types of barrier systems are currently adopted at the Mariannhill Landfill site, depending on the grade of the natural ground. On valley slopes, the barrier system consists of a stabilised sand layer onto which a geomembrane (FPP – Flexible Poly Propylene) liner and geogrid is placed. A stabilised sand protection layer is then constructed on the liner/geogrid. Crushed dump rock aggregate is then placed on this protection layer to facilitate the collection and removal of leachate.” (<http://www.durban.gov.za/durban/services/cleansing/gastoelec/landfill/fill> accessed 08/09/2008)

“In the valley basal areas, an additional component is added to the barrier system described previously. As the inflow of leachate into the strata below the landfill is critical in the valley base, two low permeability clay layers, between which a layer of 19mm stone is placed. The clay layer system is constructed below the system described previously.

The ‘sandwiched’ stone layer serves as a leachate leakage detection system, and provides further environmental protection.”

(<http://www.durban.gov.za/durban/services/cleansing/gastoelec/landfill/fill> accessed 08/09/2008)

As the waste body consists of a heterogeneous mass of material, the organic fraction of the waste begins to undergo degradation through chemical and microbiological action. This results in the production of biochemical breakdown products and the release of gases. Due to infiltration of rainfall, ground and surface waters percolating into and through the waste mass, together with the biochemical and physical breakdown, leachate is produced, which contains components of the waste, suspended solids and micro-organisms.

“At the Mariannhill Landfill site, the leachate produced from the landfill cells is collected and treated in a Sequencing Batch Reactor. This Sequencing Batch Reactor (SBR) unit is constructed of reinforced concrete 10 metres in diameter and 6 metres deep. This capacity allows for the treatment of up to 50 cubic meters of leachate daily.”

(<http://www.durban.gov.za/durban/services/cleansing/gastoelec/landfill/fill> accessed 08/09/2008)

The plant also comprises of a lined reed bed of some 280 square metres, which provides ‘polishing treatment’ for the removal of specifically residual BOD, COD and solids.

A view of the Mariannhill Leachate Treatment Plant can be seen in Plate 2.1.



Plate 2.1: Aerial view of the Mariannhill Leachate Treatment Plant

(http://www.leachate.co.uk/html/mariannhill_leachate_plant.html accessed 08/09/2008)

“All treated effluent from the SBR is fed into a balance tank, which level is controlled to supply a portion of the effluent to a standpoint for the site water tanker (dust suppression) and a portion to the reed bed. The effluent from the reed bed is used for irrigation of the vegetated areas within the conservancy area.”
 (<http://www.durban.gov.za/durban/services/cleansing/gastoelec/landfill/fill> accessed 08/09/2008)

The leachate is currently being treated for ammoniacal nitrogen removal; however most of the ammonia is being converted into nitrates through the nitrification process. The high nitrate concentration in the treated leachate exceeds the required limit of the discharge standards required by the Department of Water Affairs and Forestry (DWAF, 1998). Thus further treatment of the leachate is required to reduce the high nitrate concentrations.

2.3. The Nitrogen Cycle

Nitrogen (N_2) is the most abundant element in the atmosphere. It is an extremely stable gas and contributes approximately 78% to the Earth's atmosphere. Nitrogen is essential for many biological processes and is a crucially important component of all biological life on Earth. It is a constituent of proteins and is found in all amino acids (Tchobanoglous et al., 1985). It is also present in the bases that make up nucleic acids, such as DNA and RNA (<http://www.enviroliteracy.org/article.php/479.html> accessed 09/09/2008). However, in plants, much of the nitrogen is used in chlorophyll molecules which are essential for photosynthesis and further growth as well as in many other biological compounds (<http://www.enviroliteracy.org/article.php/479.html> accessed 09/09/2008).

The nitrogen cycle is a complex biogeochemical cycle that describes the transformations of nitrogen and nitrogen-containing compounds in nature (<http://www.enviroliteracy.org/article.php/479.html> accessed 09/09/2008). It is a cycle in which nitrogen is converted from its inert atmospheric molecular form (N_2) into a form that is useful in biological processes. This cycle is illustrated in Figure 2.2.

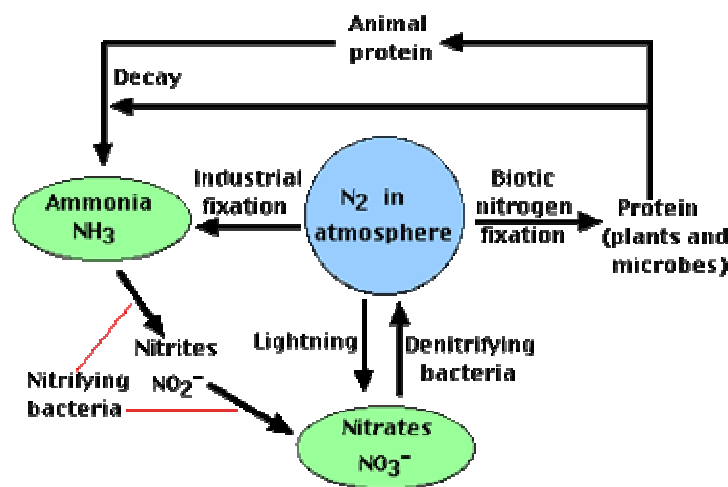


Figure 2.2: The nitrogen cycle

(<http://users.rcn.com/jkimball.ma.ultranet/BiologyPages/N/NitrogenCycle.html> accessed 09/09/2009)

Most organisms cannot use nitrogen in its inert form as a gas (N_2). Plants must thus obtain their nitrogen in a fixed form. The nitrogen needs to be converted into an organic and incorporated into compounds such as nitrate ions (NO_3^-), ammonia (NH_3) and urea ($(NH_2)_2CO$).

(<http://users.rcn.com/jkimball.ma.ultranet/BiologyPages/N/NitrogenCycle.html> accessed 09/09/2009).

The nitrogen cycle contains four processes which participate in the conversion of nitrogen in the biosphere.

(<http://users.rcn.com/jkimball.ma.ultranet/BiologyPages/N/NitrogenCycle.html> accessed 09/09/2009).

They are:

- nitrogen fixation
- decay
- nitrification
- denitrification

Nitrogen Fixation

The inert nitrogen molecule requires a considerable amount of energy to break the atoms apart so that they can combine with other atoms to form other compounds.

For nitrogen fixation in the biosphere, three processes are responsible. These include atmospheric fixation, biological fixation and industrial fixation.

(<http://users.rcn.com/jkimball.ma.ultranet/BiologyPages/N/NitrogenCycle.html> accessed 09/09/2009)

Atmospheric fixation utilises the high energy of lightning to break the atoms bonds allowing them to combine with oxygen to form nitrogen oxides (<http://www.elmhurst.edu/~chm/onlcourse/chm110/outlines/nitrogencycle.html> accessed 09/09/2008).

Biological fixation is done by micro-organisms.

(<http://www.enviroliteracy.org/article.php/479.html> as accessed 09/09/2008).

They are either bacteria living in symbiotic relationships with certain plants such as plants of the legume family, free anaerobic bacteria and algae.

(<http://www.elmhurst.edu/~chm/onlcourse/chm110/outlines/nitrogencycle.html> accessed 09/09/2008).

Industrial fixation occurs under high pressures at a temperature of 600°C with the use of a catalyst. The atmospheric nitrogen and hydrogen combine to form ammonia (<http://users.rcn.com/jkimball.ma.ultranet/BiologyPages/N/NitrogenCycle.html> accessed 09/09/2009).

Decay

The decay process is split into two procedures. These are assimilation and ammonification.

In the process of assimilation, nitrogen compounds in a variety of forms, such as nitrates, nitrites, ammonia, and ammonium are absorbed from the soil by plants via their root hairs (<http://www.enviroliteracy.org/article.php/479.html> accessed 09/09/2008). These ions are then used in the formation of plant and animal proteins. The proteins produced by plants enter and pass through the food network. At each trophic level, their metabolism produces organic nitrogen compounds that return to the environment, predominantly in the form of excretions.

(<http://users.rcn.com/jkimball.ma.ultranet/BiologyPages/N/NitrogenCycle.html> accessed 09/09/2009).

When a plant or animal dies or an animal excretes, the initial form of nitrogen in the organic matter returns to the soil. The organic matter is then converted by bacteria and other micro organisms of decay or decomposers into ammonia which is then available for other biological processes. This process is called ammonification.

(<http://www.enviroliteracy.org/article.php/479.html> accessed 09/09/2008)

Nitrification

Ammonia can be taken up directly and used by some plants usually via their roots; most of the nitrogen taken up by plants is transformed by bacteria from ammonia. This ammonia produced by decay is converted into nitrates. The conversion of the ammonia to nitrates is performed primarily by soil-living bacteria and other nitrifying bacteria.

This is accomplished in two steps.

In the primary stage of nitrification, the oxidation of ammonia (NH_3) is performed by bacteria of the genus *Nitrosomonas* species, which oxidizes and converts the ammonia (NH_3) to nitrites (NO_2^-).

(<http://users.rcn.com/jkimball.ma.ultranet/BiologyPages/N/NitrogenCycle.html> accessed 09/09/2009).

In the second stage, other bacterial species, of the genus *Nitrobacter*, oxidize the nitrites into nitrates (NO_3^-)

(<http://users.rcn.com/jkimball.ma.ultranet/BiologyPages/N/NitrogenCycle.html> accessed 09/09/2009). It is important for the nitrites to be converted to nitrates because accumulated nitrites are highly toxic to many organisms.

These two groups of autotrophic bacteria are known as nitrifying bacteria. Due to their activities nitrogen is supplied to the roots of plants. This process is known as nitrification (<http://users.rcn.com/jkimball.ma.ultranet/BiologyPages/N/NitrogenCycle.html> accessed 09/09/2009).

Denitrification

Denitrification is the process where nitrates are reduced and converted back into the inert nitrogen gas (N_2) completing the nitrogen cycle thus replenishing the atmosphere (<http://www.enviroliteracy.org/article.php/479.html> accessed 09/09/2008).

The three previous processes remove nitrogen from the atmosphere and pass it through the biosphere. This final process is performed by bacterial species known as denitrifying bacteria such as *Pseudomonas* and *Clostridium* in anaerobic conditions.

They use nitrates as an alternative to oxygen for the final electron acceptor during their respiration. This leaves free nitrogen gas as a by-product.

(<http://www.enviroliteracy.org/article.php/479.html> accessed 09/09/2008). Thus the nitrogen returns to the atmosphere and completes the cycle.

This will be discussed in more detail in section 2.6.

2.4. Human influences on the nitrogen cycle

Humans have a major influence on the environment and its natural processes. Humans have significantly contributed to and influenced the nitrogen cycle. Due to the extensive use of chemical agricultural fertilizers, increased cultivation of legumes such as soy and clover, animal feedlots, pollution emitted by vehicles and industrial plants as well as other contributing factors, mankind has dramatically increased the transfer of nitrogen into biologically available forms into the atmosphere.

(<http://users.rcn.com/jkimball.ma.ultranet/BiologyPages/N/NitrogenCycle.html> accessed 09/09/2009)

Nitric oxide (N_2O) especially has deleterious effect on the stratosphere as it acts as a catalyst in the destruction of the atmospheric ozone contributing to global warming. This contribution has also increased the transfer of nitrogen compounds into the aquatic systems (Tchobanoglous et al., 1985).

Human activities have also increased the amount of ammonia in the atmosphere. Ammonia is a reactant in the atmosphere. It acts as an aerosol, thus decreasing the air quality and adhering to water droplets.

A dramatic increase in nitrogen oxides (NO_x) to the atmosphere has also resulted from human activities, especially fossil fuel combustion. The nitrogen oxides (NO_x) alter the chemistry in the atmosphere and are the first means of ozone production in the lower atmosphere or troposphere. This increase contributes to smog, acid rain (Tchobanoglous et al, 1985) and an increase in the nitrogen input in the ecosystems. Acid rain is formed when nitrogen oxides (NO_x), released into the atmosphere, react with water, oxygen, and other substances to form a mild solution of nitric acid. (<http://environment.nationalgeographic.com/environment/global-warming/acid-rain-overview/> accessed 18/02/2010).

Wastewater also has a major influence on the nitrogen in the environment. Nitrogen from wastewater discharges and onsite sewage facilities such as septic tanks may enter the aquatic systems by either being discharged directly into streams or through groundwater discharge (Tchobanoglous et al., 1985).

The development of landfills has also influenced the nitrogen cycle. These landfills effect the cycle through decomposition of organic matter and thus the production of biogas and leachate which have high concentrations of ammonia. The leachate enters the water systems by percolating through the soil into the groundwater flow (Tchobanoglous et al., 1985).

2.5. The effects of nitrogen and its compounds

Nitrogen and its compounds can have a negative effect on the environment. The two main effects are that of over enrichment problems or eutrophication as well as increasing the biochemical oxygen demand of the natural water systems (Tchobanoglous et al., 1985).

Eutrophication is the process where by algae growth in natural water bodies is accelerated due to excessive nutrients entering the system. The nutrients such as nitrogen and its compounds stimulate plant growth. The plants and algae can use nitrates directly as their nitrogen source. Thus releasing excessive amounts of nitrates into the surface waters is compared to supplying a fertilizer to the system (Tchobanoglous et al., 1985). Due to the added nitrogen, algae growth can increase to a damaging level. This is also referred to as an algae bloom. The high algae concentration blocks out and prevents sunlight from entering the water body, which is essentially needed by other aquatic plants and species. This creates an anaerobic environment under the water surface (Tchobanoglous et al., 1985). Thus the plants use the oxygen in the water creating an oxygen deficit. This oxygen is required by all marine life. Therefore this depletion of oxygen levels in the water kill off aquatic life. Eutrophication is a natural process which occurs slowly over time; however human activities have influenced the process by causing an acceleration in the process. This causes an imbalance in the ecosystem.

(<http://users.rcn.com/jkimball.ma.ultranet/BiologyPages/N/NitrogenCycle.html> accessed 09/09/2009)

Nitrate Contamination

The contamination of drinking water due to the presence of nitrogen and nitrates is a major concern. Although nitrates are found in most natural water systems, at low concentrations, elevated levels in the groundwater are problematic. This groundwater often ends up being used as drinking water.

Nitrogen and nitrates are introduced into the water systems by means of wastewater, both municipal and industrial, animal wastes, leachate from the decomposition of organic matter decaying in the ground such as landfills, overflow or run off from septic tanks as well as by chemical fertilizers which are used in agriculture.

These fertilizers and wastes are all sources of nitrogen containing compounds which are converted into nitrates in the soil. Nitrates are highly soluble in water and thus move through the soil easily into the drinking water supply.

Excessive amounts of nitrate are harmful (<http://www.epa.gov/safewater/dwh/c-ioc/nitrates.html> as accessed 09/09/2008). At high concentrations they threaten the health of infant animals, as well as humans, through nitrate poisoning. Due to the lower acidity in an infant's intestinal tract, the growth of nitrate reducing bacteria which converts nitrates to nitrites is permitted. Thus the nitrites are absorbed into the bloodstream. As their digestive systems are not capable of transforming the nitrates into less harmful forms of nitrogen, thus once the nitrates enter the infant's bloodstream they interfere with oxygen transfer, as nitrites have a greater affinity for haemoglobin than does the oxygen. Thus the nitrite replaces the oxygen in the blood complex (Tchobanoglous et al., 1985). The body is thus denied the essential oxygen required and is thus starved of the oxygen it needs. The casualty thus suffocates. As a result of this oxygen starvation, a bluish discolouration of the skin and body occurs in the victims. This nitrite poisoning is often referred to as the "blue baby" syndrome or correctly known as methoglobinemia (Tchobanoglous et al., 1985). However once the flora of the intestinal tract has fully developed, usually after the age of about six months, the problem of methoglobinemia is reduced (Tchobanoglous et al., 1985).

Long term effects of exposure to high levels of nitrate containing water are diuresis, increased starchy deposits and haemorrhaging of the spleen.

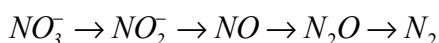
The combination of nitrates with amines, amides, or other nitrogenous compounds through the action of bacteria in the digestive tract results in the formation of nitrosamines, which are potentially carcinogenic (Mekonen et al., 2001).

Due to the health risks associated with increased levels of nitrates in drinking water, it was established by United States Environmental Protection Agency (EPA) as well as according to the World Health Organization (WHO) guidelines and recommendations, that a maximum contaminant level (MCL) allowable for nitrate concentration is 10 ppm or 10 milligrams per litre be set. The Department of Water Affairs and Forestry (DWAF) also uses a limit of 10 milligrams per litre for nitrates in drinking water.

2.6. Denitrification

Denitrification is commonly defined as the process in which nitrate (NO_3^-) is converted into di-nitrogen gas by means of intermediate products including, nitrite, nitric and nitrous oxide (Haandel et al., 1981; Platzner, 1999; EPA, 1998). From the biochemical perspective, bio-denitrification is a *bacteria-mediated* process where by nitrogen oxides, in both gaseous and ionic forms, such as nitric and nitrous oxide, act as terminal electron acceptors as a means of electron transport. These electrons are carried from an electron-donating substrate, through numerous carrier systems to a greater oxidized form of nitrogen.

The different denitrification steps are presented below (Mateju et al., 1992)



The reaction is irreversible and occurs in the presence of an available organic source under either anaerobic or anoxic conditions ($E_h = +350$ to $+100$ mV) where nitrogen is used as an electron donor in the place of oxygen (Trois et al., 2010).

Influencing factors on the denitrification process include the absence of oxygen, thus dissolved oxygen (DO), redox potential, which is an electrical measurement of the affinity of a substrate for electrons, temperature, pH value, presence of denitrifiers carbon source and nitrate concentration.

The presence of oxygen inhibits nitrate reductase, which is the enzyme which ensures the electron transport in the denitrification process. In the presence of both oxygen and nitrogen compounds (nitrate), the aerobic process will be favoured.

Denitrification is highly dependant on temperature. The rate of denitrification increases up to maximum temperatures between 60 and 75°C and decreases rapidly thereafter. (Paul and Clark, 1996). At low temperatures, below 5°C, denitrification occurs at a much slower rate (Van Oostrom and Russell, 1994).

pH is a limiting factor in the denitrification process and thus low pH values will impact negatively on the rate of nitrate removal, at the optimum pH range for biological denitrification is between 6 and 8 as noted by Paul and Clark (1996) and Trois et al. (2007). The rate of denitrification is reduced at a pH below 5; however if the pH drops lower than 4, denitrification is negligible to absent. It is also important to note that the denitrification process produces alkalinity (Paul and Clark, 1996).

The rates of denitrification are dependent on both the carbon and energy sources used as well as on the carbon to nitrogen (C/N) ratio. As stated by Bandpi and Elliot (1998), low carbon to nitrogen ratios can cause the accumulation of nitrites, while the dissimilative reduction to ammonium can occur at high carbon to nitrogen ratios (Gylsberg et al., 1998) both of which are detrimental to the denitrification process.

There is little information regarding inhibitors to bio-denitrification. However, it has been found, that some types of bacteria are adversely affected by certain substances, for example some species of nitrate reductase enzymes are sensitive to, cyanide, bivalent copper and some mercury compounds (Carucci, 2003).

There are various treatment methods used for nitrate removal in waste waters. These can be separated into two main treatment processes: physico–chemical and biological methods.

The most conventional abiotic or physico–chemical treatment processes include reverse osmosis, active carbon adsorption, ion exchange, electro-dialysis amongst other advanced oxidation processes (Mateju et al., 1992; Islam and Suidan, 1998; Ergas and Reuss, 2001; Shrimali and Singh, 2001; Feleke and Sakakibara, 2002; Prosnansky et al., 2002).

There are, however, some disadvantages to these conventional methods, which limit their implementation in full scale applications as a result of their operation costs, long term maintenance and the disposal of by-product such as brine etc.

Some methods tend not to be ion specific (Mateju et al., 1992) and result in the transfer of only the pollutants in concentrated solution or adsorption on solids without solving the specific environmental problems (Christensen et al., 1992). The ion exchange process removes both nitrate and sulphate simultaneously; however wastewater is produced from the resin regeneration process (Shrimali and Singh, 2001). Although the reverse osmosis treatment process is able to separate and concentrate nitrates contained in water without changing their molecular structure, its application is limited due to the high costs and the production of concentrated waste brine which poses a disposal problem (Ergas and Reuss, 2001; Shrimali and Singh, 2001).

Biological denitrification processes seem to be a more robust and versatile treatment approach, compared to abiotic methods, which are often unable to completely separate or remove nitrates from the effluent resulting in the production of problematic by-products (Shrimali and Singh, 2001).

Bio-denitrification refers to the mechanism by which inorganic nitrogen compounds such as nitrite and nitrates are converted into nitrogen gas microbially by denitrifying bacteria, so that no further treatment is required. The denitrifying bacteria use nitrate as an electron acceptor in their respiratory process in the absence of oxygen. The microbial removal of nitrates from polluted water and wastewaters seems to be the most viable strategy as it is both cost effective and environmentally friendly (Soares, 2000). The only drawback of biological denitrification may be due to the slower rate of removal at high nitrate concentrations (Foglar et al., 2005).

The microbial conversion utilized in biological denitrification can occur through two mechanisms: the assimilation route and the dissimilation route, also known as the “dissimilatory nitrates reduction” (Pelmont, 1993).

The assimilation route involves the reduction of nitrates to ammonia, which become the direct source of nitrogen assimilated by an indigenous microbial community. The dissimilation route however, is a respiration process, whereby micro organisms use an external organic substrate as an electron donor instead of oxygen to reduce nitrates (Ovez, 2006).

The main stage in the reduction of nitrates into nitrites, in both the assimilation and dissimilation routes, is carried out by an enzyme, referred to as nitrate reductase. This stage differs according to the route taken. In aerobic conditions, bacteria prefer to use oxygen as an electron acceptor rather than nitrates. Although nitrate reductases are still produced, these enzymes are not used in the process and denitrification is thus inhibited (Pelmont, 1993).

In the dissimilatory nitrate reduction process, the nitrate reductase is of vital importance and ensures the electron transfer to occur. Nitrates serve as terminal electron acceptors for respiration instead of oxygen, thus generating energy that can be used to produce new cells and maintaining existing ones (Ovez et al., 2006).

Biological denitrification is often performed by facultative anaerobes, which require organic and inorganic sources for food and energy. The denitrifying micro-organisms involved in the dissimilatory nitrites reduction can be classified into two main groups: heterotrophic, which utilise complex organic substances as a carbon source and autotrophic, that utilise hydrogen and carbon dioxide or reduced sulphur compounds. Denitrifying bacteria are usually heterotrophic, that favour the dissimilatory route with the production of nitrate reductase. Denitrifiers are part of various genera such as *Achromobacter*, *Alcaligenes*, *Bacillus*, *Corynebacterium*, *Paracoccus*, *Pseudomonas*, *Spirillum*, *Thiobacillus*, *Xanthomonas*. All these aerobic species of bacteria are able to use nitrates whenever oxygen is absent for their respiration process and as such are called facultative anaerobes (Pelmont, 1993; Mateju et al., 1992)

Denitrifiers, however, differ widely with selected substrates, thus further studies are required to determine the bacteria associated with the specific substrate chosen (Costa et al., 2000). Microbiological and bio-chemical studies are important to obtain a greater understanding of the complex processes and ideal environmental conditions for efficient and productive growth of microbial populations associated with denitrification. Each microbial population has its own preferential environmental conditions: e.g. concentration and type of the substrate, nutrient concentration, size of the system, temperature, pH, mixing of the system, level of oxygen, inhibitory substances and process type (Metcalf & Eddy Inc., 1991). Therefore the design of a reactor must be in accordance with those environmental conditions in order to enhance reaction rate and efficiency (Sundaram et al., 2008).

As previously discussed, denitrification occurs primarily under anaerobic and/or anoxic conditions. The pre-treated effluents produced from secondary treatment plants, contain very low concentrations of easily biodegradable organic matter (Spagni et al., 2007) thus an external carbon and energy source is required to enable biological denitrification to be accomplished.

Carbon sources include sucrose, methanol, ethanol, propionate or acetic acid (Mohseni-Bandpi et al., 1999; Gomez et al., 2000), methane (Zhong et al., 2009, Modin et al., 2007), or molasses (Najafpour et al., 2003). However these carbon sources are not a viable solution for developing countries, due to their high cost and high-energy requirements and, are generally not suited for large scale field applications (Tsui et al., 2007; Volokita et al., 1995). Solid carbon sources such as tree bark, wood chips, corncobs, newspaper, sawdust and compost appear to be a more appropriate solution (Tsui et al., 2007; Volokita et al., 1995).

Gomez et al. (2000) compared the efficiency of sucrose, ethanol and methanol as carbon sources for denitrification of contaminated groundwater. The variance of process yields, biomass production, nitrate accumulation and the growth of denitrifying bacteria were investigated (Gomez et al., 2000). They found that technology based on submerged filters appears to have a better applicability for freshwater biological treatment than alternatives such as rotating biological contactor, moving bed or fluid bed configurations (Gomez et al., 2000). Data analysis suggested sucrose to be the least efficient of the three carbon sources with ethanol being considered the most suitable since methanol, although efficient, is toxic (Gomez et al., 2000).

2.7. Traditional treatment systems using chemicals as carbon sources

Modin et al. (2007) investigated the merits of methane as “a potentially inexpensive, widely available electron donor for biological denitrification of waste water, landfill leachate or drinking water.” Important reference is made to the onsite generation of methane resulting from anaerobic process in respect of sludge in waste water and landfill organic waste degradation making it imminently suitable in these circumstances as a carbon source (Modin et al., 2007).

Previous studies in respect of methane as an electron donor were carried out by Costa et al. (2000). The study concluded that the occurrence of denitrification under reduced oxygen circumstances in respect of methane acting as an electron donor appears to arise out of a “consortium of methanotrophic and denitrifying bacteria”, postulating that acetate is produced by the methanotrophs as the compound responsible for electron donation (Costa et al., 2000).

In respect of the various treatment methods available for nitrate removal, which include ion exchange, reverse osmosis, electro-dialysis, distillation, chemical denitrification and biological denitrification, there appears to be general consensus in the literature that the biological processes have proved to be practical, efficient and most importantly cost effective (Mohseni-Bandpi et al., 1999). Among the biological systems, the most widely used are Sequencing Batch Reactors (SBR).

Sequencing batch reactors

The sequencing batch reactor (SBR) is a fill and draw activated sludge system for the treatment of wastewater (EPA, 1999). The system is designed to operate as a single “batch” reactor under non-steady state conditions to treat and remove detrimental components from wastewater prior to being discharged (<http://www.rpi.edu/dept/chem-eng/Biotech-Environ/Environmental/Steps/EnvSysSBR.html>; EPA, 1999).

The sequencing batch reactor allows equalization, aeration, sludge settlement and clarification to occur in a single reactor. The SBR tank carries out these processes in a time sequence lasting approximately 24 hours. This system has been successfully utilized to treat both municipal and industrial wastewater (EPA, 1999).

The process involved in an SBR begins with the screening of influent wastewater prior to entering the reactor. This wastewater is added to acclimated biomass with elements of the wastewater. The system is aerated and mixed, until the suspended biomass is able to achieve the biological reactions. Once finished, the biomass is allowed to settle and the treated effluent is removed. This technology is founded on the suspended growth, as bacteria are mixed and suspended simultaneously.

The main advantages of the system are as follows: a single reactor is utilized to achieve equalization, clarification and biological treatment, whilst the operating conditions are both flexible and easily controlled.

The main drawback to this system is the high degree of sophistication which leads to both greater levels of maintenance and the associated increased costs.

Fernandez-Nava et al. (2008) studied nitrate removal from waste water produced in the stainless steel manufacturing process. The investigation tested two different inocula. Sludge from the biological treatment of leachate emanating from a municipal solid waste landfill and sludge from a sewerage treatment plant (Fernandez-Nava et al., 2008). The influences of calcium concentration and COD/N ratio were investigated. A sequential batch reactor (SBR) employing methanol as a carbon source was used in the study because such reactors are robust, occupy less space and they are “more efficient in recovering biomass, they facilitate the change in scale and have been shown to be effective in high nitrate wastewater denitrification processes” (Fernandez-Nava et al., 2008). It was found that “prior acclimation of the sludge to high nitrate concentrations increases the denitrification rate” (Fernandez-Nava et al., 2008) while the presence of calcium in the water proved to be an impediment.

The study concluded that biomass emanating from landfill leachate treatment plants allowed successful denitrification to levels acceptably below established discharge limits (Fernandez-Nava et al., 2008).

The efficiency of the sequential batch reactor was also tested by Mekonen et al. (2001) who found it to be effective in a study in which ethanol was used to reduce nitrate concentrations in drinking water to acceptable levels.

Mohseni-Bandpi et al. (1999) conducted their investigation using a pilot scale SBR. The study considered the determination of the acetic acid to nitrate-nitrogen (A/N) ratio, the effect of influent nitrate-nitrogen concentration, denitrifying bacteria and effluent quality, confirming the suitability of using acetic acid as a carbon source to achieve 83% to 98% removal efficiency rate for the reactor (Mohseni-Bandpi et al., 1999).

2.8. Organic Carbon Sources

Investigations have been carried out in respect to organic carbon sources.

Volokita et al. (1995) investigated the efficiency of microbial denitrification of drinking water, conducting a laboratory study using columns with shredded newspaper “as the sole carbon and energy substrate” (Volokita et al., 1995).

The investigation is of particular significance as nitrate contamination of drinking water as well as natural water systems is increasingly prevalent in developed countries in addition to the developing world, as a result of the “excessive use of fertilizers” (Volokita et al., 1995) which is an endemic phenomenon associated with intensive agricultural practice. Contamination is exacerbated by the tendency to utilise ammonia-rich effluents from wastewater treatment plants to supplement irrigation (Volokita et al., 1995).

In general, effective removal of nitrates from drinking water on a large scale is inhibited by high costs associated with some processes and consequently non compliance in respect of the W.H.O. and other benchmarks are not uncommon especially in countries experiencing fiscal challenges.

Under oxygen starvation, aerobic bacteria will revert to accepting nitrate as a terminal electron donor in respiration and consequently it is of significance that anaerobic conditions are instigated (Payne, 1981).

Volokita et al. conducted investigation of 0.4 cm shredded newspaper packed in 55cm PVC columns subjected to a nitrate amended tap water feed regulated by peristaltic pumps (Volokita et al., 1995). Significantly according to Volokita et al. “complete removal of nitrate without accumulation of nitrite was achieved after the onset of flow (0.55 m/d)” (Volokita et al., 1995), where the flow used is in terms of metres per day (m/d). Day 19 observation indicated a temporary nitrate breakthrough which was attributed to be due to the sharp drop in temperature recorded as 6° - 10°C during this period (Volokita et al., 1995).

Evidence indicates that flow rate appears to be a critical factor in maintaining stable denitrification. Increases in flow rate to 0.77 m/d and 0.95 m/d resulted in a breakthrough of nitrate (up to a concentration of 54 mg/ℓ) and a low concentration of nitrite. “Upon decrease of flow rate to 0.4 m/d (day 148) nitrate and nitrite disappeared from the effluent”. No ammonia was detected during the study (Volokita et al., 1995).

Study concluded that there is evidence to suggest that newspaper, as a cheap and readily available carbon source, has effective capacity in respect of quick denitrification of low level nitrate contaminated water sustainable over a significant time period.

The absence of detectable colour, odour or flavour in the treated effluent is an additional endorsement in respect of newspaper as a substrate although a high level of bacteria washout and further disinfection is required in the process (Vokokita et al., 1995).

Diaz et al. (2003) conducted an investigation to develop an experimental method for nitrate removal from secondary effluents. The study identified three plant substrates namely pine bark, almond shells and walnut shells as pertinent organic sources and used gravel as the control medium.

Comparison investigations were conducted utilising eight cylindrical open air batch reactors as an alternative to a rectangular open air continuous flow device (Diaz et al., 2003).

Denitrification of urban municipal waste water was measured considering the variance of hydraulic retention time, water temperature and, in respect of the batch reactors, influent nitrate concentration (Diaz et al., 2003).

Data analysis confirmed denitrification occurring in all three substrates and that nitrate removal was seen to be dependent on the variants chosen.

Diaz et al. (2003) proposed that effectiveness of each substrate was linked to its biodegradability and furthermore; that the continuous flow reactor proved to be the more efficient device on the postulation that water circulation favoured the rate of organic matter release and dispersion. The study details methodology, characterisation of the organic matter released by the plant substrates, carbon and nitrogen composition and lasting properties of the substrates as well as specification of the continuous flow reactor nitrate removal process (Diaz et al., 2003).

Diaz et al. (2003) concluded that data produced by their study indicated that all three substrates were suitable for nitrate removal, that the volumetric nitrate removal rates were well above those observed in reactors operating with wetland sediments although lower than those in conventional rotating biological systems. All three substrates had good lasting properties and that the system tested provided a promising alternative particularly in terms of energy and consequently cost saving as well as operational and maintenance simplicity (Diaz et al., 2003).

In respect of the feasibility of using immature compost as a substrate Tsui et al. (2007) presented a preliminary assessment of the suitability of immature compost for the denitrification of tile drainage water based on its relatively large organic content, high microbial activity and pH buffering capacity. Tile drainage is a method used to remove excess water from the subsurface of soil. It consists of a network of pipes below the ground that allow subsurface water to move out from soil used for both agricultural as well as urban run-off.

The high cost of easily biodegradable carbonaceous materials such as glucose, methanol, ethanol, propionate or acetic acid as well as their high solubility reduces their suitability for agricultural application (Tsui et al., 2007).

The consideration of other solid materials, including tree bark, wood chips, corn cobs, newspaper and sawdust were questioned by Tsui et al. (2007) who suggested their relative inert nature would reduce their efficiency to denitrify surface runoff which commonly occurs after rainfall (Tsui et al., 2007).

Tsui et al., (2007) postulate that compost, as a result of its high microbial activity, and in particular immature yard waste which has larger carbon content could prove to be a more viable carbonaceous source for denitrification in the agricultural context (Tsui et al., 2007). These assertions were tested using six month old compost samples collected from the Urbana Landscape Recycling Centre in Illinois. Compost sample characterisation, batch extraction, effect of compost mass on denitrification as well as the effect of flow rate in compost storage as factors relating to nitrate removal were investigated (Tsui et al., 2007).

Adequate data analysis as presented by the study indicated that composted material satisfying the identified selection parameters “demonstrated a significant potential as a bioreactor medium to remove nitrate from solutions” (Tsui et al., 2007). They were able to conclude that the extent of denitrification would be regulated by hydraulic retention time but not necessarily by compost mass (Tsui et al., 2007). These encouraging results provide a preliminary platform for further study which should be focussed on bioreactor packing processes and an investigation into optimal compost storage procedures.

2.9. Simulation of Fixed Bed Reactors using Column Studies

The development of applicable, economical, easily implementable strategies based on an environmental model are the most viable option in respect of successful landfill leachate treatment in South Africa (Trois, Strachan and Olufsen, 2002). Current South African legislation in line with judicial authority elsewhere prohibits the deliberate discharge of contaminated waters into natural wetlands regulating such wetlands as a buffer for “diffuse source pollution” and subject to “stringent” standards (Wetzel, 1993; Reed et al., 1995; Rogers et al. 1985 and Olufsen, 2003). Of concern is the current avenue of dissemination which permits direct discharge of leachate into the sewer line. Such practice, if excluding risk management of the explosive potential associated with dissolved methane, as well as the tendency to regard dilution as a sufficient process should be discouraged (Trois, Strachan and Olufsen, 2002).

Clearly there is a growing awareness that ideally, contamination should be treated at source with cost effective technology. Such technology should be suitable for use in urban as well as rural environments which typically are remote and certainly under-resourced in respect of access to infrastructure (Trois, Strachan and Olufsen, 2002).

Typical fixed bed reactors used around the world for the treatment of leachate are Constructed Wetlands and, in particular, the Subsurface Flow Constructed Wetland (SF CW) (Trois, Strachan and Olufsen, 2002; Kadlec and Knight, 1996 and Nivala et al., 2006).

Subsurface Flow Constructed Wetlands have been instigated on a pilot scale at the Jones County Municipal Landfill near Anamosa Iowa to demonstrate their viability as a low-cost, effective treatment option for landfill generated leachate (Nivala et al., 2006). The study was prompted by the call for better comprehension of “transformation movement and treatment” of contaminants in such systems (Mulamoottil et al., 1998). The investigation identifies the promising potential of subsurface flow constructed wetlands in terms of landfill produced leachate treatment. The study cites a number of appealing factors including the “small ecological footprint” associated with such wetlands, their utilisation of low-level technology and their possible “aesthetic value” being construed to assimilate that of natural wetlands (Kadlec and Knight, 1996 and Nivala et al., 2006).

S.C. Reed et al. (1995) postulate that a constructed wetland should typically exhibit greater efficiency in comparison with a natural wetland of equal area. This could be attributed to the fact that constructional parameters permit selection of appropriate composition, material and design, thus enabling control of biological factors, flow rate and “hydraulic regime” (Reed et al., 1995). It is, thus, possible to eliminate the extreme variability associated with the functional components of natural wetland which inhibit accurate prediction responses to contaminated water applications into such systems (Reed et al., 1995; Brix, 1993).

Importantly, the use of a constructed wetland circumvents the legislative restrictions governing natural systems (Brix, 1993). It is possible to improve process reliability by managing the vegetation and other system components, if and when needed (Reed et al., 1995). Furthermore, performance improvement could be effected by the application of modification if necessitated (Reed et al., 1995). Finally the system lends itself to abandonment and assimilation into the environment in the eventuality of the need ceasing to exist (Reed et al., 1995).

Construction parameters in the Subsurface Flow Constructed Wetland (SF CW) are underpinned and conform to the principle of the fixed bed reactor. The fixed bed reactor is a well-known, efficient device for carrying out chemical and biological reaction processes primarily regulated by a catalyst (usually solid) packed in a bed located in a fixed position.

Fixed bed reactors have several favourable features. They are typically simple in design. The absence of moving parts in the device significantly reduces operational wear and tear and the catalyst is confined and contained in the reactor. The fixed bed reactor employs a continuous flow system enabling regulation and control of the appropriate flow rate. Reaction is facilitated as the reactant passes through the catalyst at the desired rate.

In this research column studies were set up to accurately simulate fixed bed reactors (Tsui et al., 2007; Diaz et al., 2003; Volokita, 1995) and consequently subsurface flow constructed wetlands (Gomez et al., 2000).

This simulation was chosen for the purpose of investigation and prediction of the optimal flow rate required to effect efficient denitrification based on substrate reaction kinetics and hydraulic retention time.

Such prediction will assist in respect of the preferred subsurface flow constructed wetland choice.

Two varieties are under consideration. The horizontal subsurface flow constructed wetland as seen in Figure 2.3, also referred to in the literature as vegetative submerged bed (VSB) (Olufsen, 2003) and the vertical flow subsurface flow wetland or VF shown in Figure 2.4.

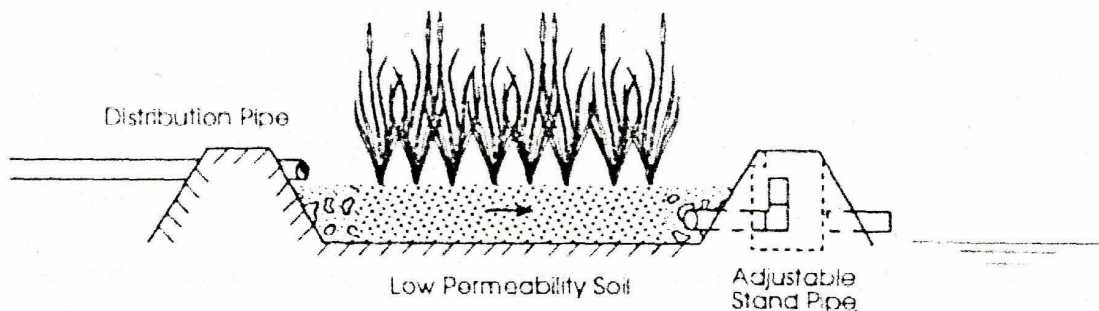


Figure 2.3: Typical cross sectional layout of a VSB (Kadlec and Knight; 1996)

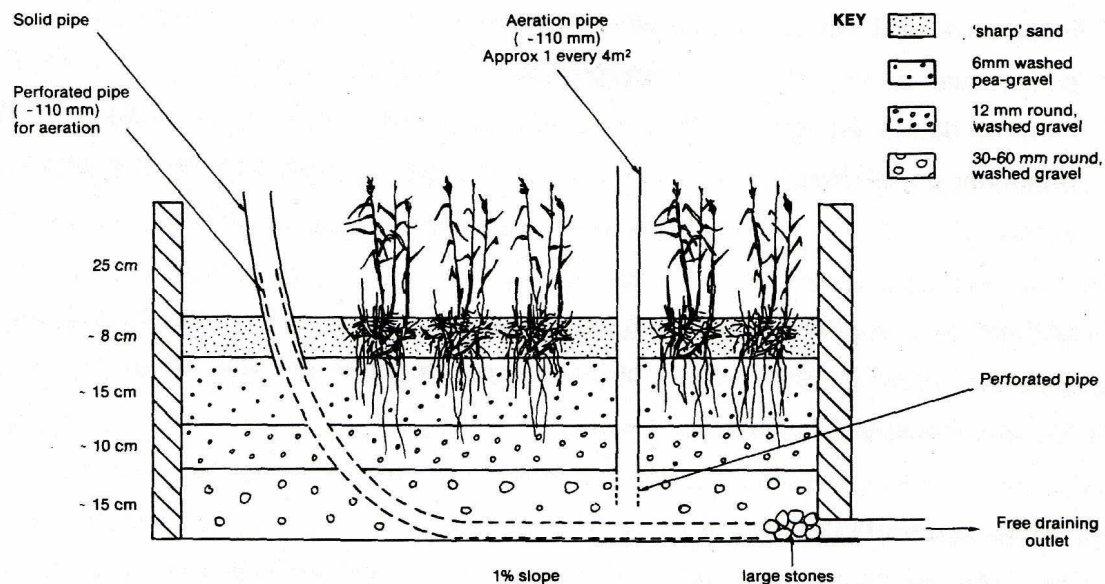


Figure 2.4: Typical cross sectional layout of a VF constructed wetland (EC/EWPCA, 1990)

The two systems share design features in that both are evacuated basins filled with substrate such that the free water level may be maintained at or below the top of the substrate in order that the water is not exposed to the atmosphere (Olufsen, 2003).

The systems may be planted with suitable, “emergent aquatic vegetation” and lining of the basin is recommended (where applicable) to inhibit and prevent contaminated aqueous seepage into the groundwater and consequent damage to the surrounding ecosystem (Kadlec and Knight 1996; Olufsen, 2003 and Reed et al., 1995).

The systems are distinguishable from each other in that, in the former, influent flow occurs horizontally through the medium emerging typically from an adjustable stand pipe (Kadlec and Knight, 1996 and Olufsen, 2003) whereas in the latter, application of polluted water occurs vertically, perforated piping is employed and effluent emerges by way of a free draining outlet (Kadlec and Knight, 1996; Brix, 1993; Reed et al., 1995 and Olufsen, 2003).

CHAPTER 3

3. MATERIALS AND METHODS

3.1. Introduction

This investigation was aimed at studying and identifying an efficient, cost effective and feasible natural organic carbon resource such as pine bark, raw and composted garden refuse. These organic substrates contain relatively high amounts of carbon and are readily available in the major eThekweni landfills.

The substrates investigated included fresh pine bark (PB) and fresh commercial garden refuse (CGR RAW), immature domestic garden refuse (DGR 10) and commercial garden refuse (CGR 10) compost (treated for 10 weeks) and mature CGR compost (treated for over 4 months). The mature compost used two different composting techniques e.g. the Dome Aeration Technology (DAT) (Trois et al., 2007; Paar, 1999a; b; Mollekopf et al., 2002) and traditional turned windrows.

The suitability of the above substrates as carbon sources for denitrification was assessed using small scale batch tests and larger scale columns.

The initial stage of the experimentation was to comprehensively characterise the substrates through conventional testing done on both the solid substrates and their eluates.

The batch tests were conducted at 3 different nitrate concentration levels: 100, 500 and 2000 mg NO₃/ℓ. A blank test (0 mg NO₃/ℓ) was conducted using distilled water for each substrate. A synthetic nitrate solution was used to simulate the leachates produced from Mariannhill Landfill site so as to prevent any disturbances in the nitrate (NO₃) analysis due to the presence of chlorinated compounds in the leachate as experienced by Pisano (2007) in previous studies.

Three substrates were then selected for the large scale experiments in columns due to their biodegradability and availability of carbon in particular reference to the carbon to nitrogen ratio, Respiration Index as well as their performance in terms of denitrification.

The substrates chosen were the fresh pine bark (PB), the fresh CGR (CGR RAW) and the immature CGR compost (CGR 10).

Two nitrate concentrations (500 and 2000 mg NO₃/ℓ) and two different flow rates were used for the column campaign. Each experiment was performed over 4 weeks, with the second experiment being prolonged to ascertain the affect the previous flow rates had had on the substrates. The columns were thus left for a further week in flooded conditions.

Durability tests were conducted on previously used substrates of pine bark and immature compost to determine the period for which the substrates could be used as a means for denitrification before replacement was necessary.

Microbial analysis was conducted by De Combret (2009) during the research in order to monitor and assess the affect of the different substrates on the indigenous bacteria population. Batch tests were conducted at a nitrate concentration of 500 mg/ℓ for three different substrates. The growth of the microbial community was followed using a spread plate enumeration technique; the colonisation of the substrates was assessed through Environmental Scanning Electronic Microscopy (ESEM), and an insight into the composition of the bacterial community was determined by phylogenetic analysis (Trois et al., 2010). Findings of the work can be seen in Appendix E.

The testing was conducted in the Environmental Engineering laboratory at the University of KwaZulu-Natal. Some of the testing was carried out by BemLab in the Western Cape and Stewart Inspection and Analysis situated in Durban. The standard test procedures published by “Standard Methods for the Examination of Water and Wastewater” Clesceri et al. (2005) were followed.

The complete research framework followed can be seen in Figure 3.1

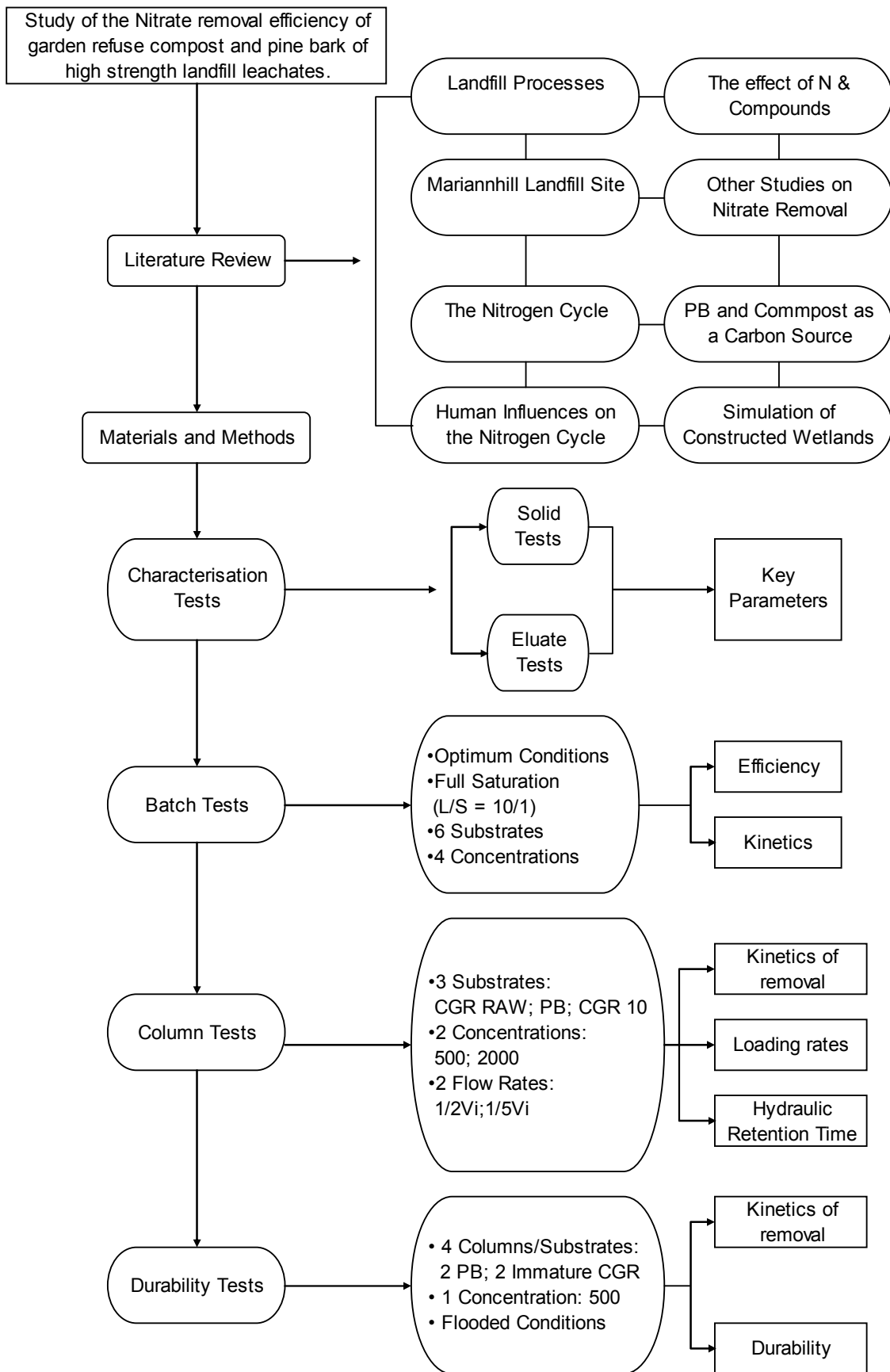


Figure 3.1: Research framework

3.2. Materials

This investigation involved the denitrification of treated landfill leachate using organic carbon sources. The leachate was simulated using a synthetic solution so as to operate the denitrification process in controlled conditions and to eliminate the disturbances in the nitrate (NO_3) analysis due to the presence of chlorinated compounds in the leachate, as experienced in previous studies (Pisano; 2007). The substrates investigated in the research were garden refuse and pine bark at different levels of stability and maturity (Gomez, 2006; Adani et al., 2006; Adani et al., 2001): fresh pine bark (PB) and fresh commercial garden refuse (CGR RAW), immature domestic garden refuse (DGR 10) and commercial garden refuse (CGR 10) compost (composted for 10 weeks using forced aeration) and mature CGR compost, treated for over 4 months using two different composting techniques e.g. the Dome Aeration Technology (DAT) (Trois et al; 2007, Paar, 1999a; b; Mollekopf et al., 2002) and traditional turned windrows.

3.2.1. Synthetic Nitrate Solution

A synthetic nitrate solution was used to simulate the treated leachate produced from a Sequencing Batch Reactor (SBR) at the Mariannhill Landfill Site. Three different concentrations of nitrate (NO_3) solution were utilised during the investigation. The nitrate (NO_3) concentrations used were 100, 500 and 2000 mg NO_3/ℓ . These concentrations were chosen as a result of the typical ranges of nitrate concentrations displayed by the treated landfill leachate produced by the Sequencing Batch Reactor (SBR) at the Mariannhill Landfill Site as seen in Tables 3.2 and 3.3.

Table 3.1: Typical elements and compound atomic weights

Elements	Atomic Weights
Potassium (K)	39.098
Nitrogen (N)	14.007
Oxygen (O)	15.999
Potassium Nitrate (KNO_3)	101.102
Nitrate (NO_3)	62.004

Table 3.2: Mariannhill treated landfill leachate

Date	Nitrate + Nitrite (mg N/ℓ)	Nitrate + Nitrite (mg NO ₃ /ℓ)	pH	COD (mg/ℓ)
02/02/2009	121.0	535.6	7.60	761
26/02/2009	91.0	402.8	8.00	800
09/04/2009	119.0	526.8	7.80	545
29/04/2009	478.0	2115.9	7.40	1254
26/05/2009	111.0	491.4	7.49	839
01/07/2009	2.0	8.9	7.92	2329
06/10/2009	2.8	12.4	7.35	912
27/10/2009	3.2	14.2	7.73	762

Table 3.3: Summary of Mariannhill treated landfill leachate and synthetic solution

Parameter	Minimum	Maximum	Average
Nitrate + Nitrite (mg N /ℓ)	2.0	478.0	478.0
Nitrate + Nitrite (mg NO ₃ /ℓ)	8.9	2115.9	513.5
pH	7.4	8.0	7.7
COD (mg/ℓ)	545.0	2329.0	1025.3
Synthetic Solution			
Nitrate Concentration (mg NO ₃ /ℓ)	100	500	2000

3.2.2. Pine Bark

A large quantity of pine bark is produced every day at the SAPPI (South African Pulp and Paper Industry) paper mills around the country. The trees grown by SAPPI are mainly of the *Pinus patula* variety. A large portion of botanists are of the opinion that the genus *Pinus* contains two subgenera.

(<http://www.britannica.com/EBchecked/topic/460904/pine#ref274395> accessed 3/12/2009). The soft pines or *Haploxylon*, have one fibrovascular bundle whereas the hard pines, *Diploxylon*, with rough and fissured branches and young stems have two (<http://www.britannica.com/EBchecked/topic/460904/pine#ref274395> accessed 3/12/2009). The typical activities at a SAPPI's mill begins at the woodyard, when timber, in the form of both logs and chips, is received and then debarked and chipped. The chips are then stored in large piles (<http://www.engineeringnews.co.za> as accessed 3/12/2009).

The pine bark used in this research is from the tissue/cells outside of the vascular cambium of the hard pine, *Diploxylon* tree, which is responsible for forming the wood and inner bark of the tree.

(<http://www.britannica.com/EBchecked/topic/460904/pine#ref274395> accessed 3/12/2009).

Some of the pine bark is disposed of at local landfill sites as well as SAPPI's disposal facilities. The pine bark used in this investigation was collected, fresh, from SAPPI within 24 hours of debarking. A sample of fresh pine bark can be seen in Plate 3.1.



Plate 3.1: Fresh pine bark substrate

3.2.3. Garden Refuse

For the research, fresh garden refuse and composts of different stages were used.

1. Fresh commercial garden refuse (CGR RAW).

A large amount of garden refuse is disposed of at both the Mariannhill and Bisasar Road Landfill sites in Durban separated from the main waste stream. Commercial garden refuse consists mainly of branches and plant trimmings from parks and green municipal areas. At the Bisasar Road Landfill, the CGR is passed through a chipper to reduce the particle size to approximately 4 – 5cm length and then composted. The CGR sample was collected from the landfill soon after the size reduction phase. A sample of fresh commercial garden refuse can be seen in Plate 3.2.



Plate 3.2: Fresh commercial garden refuse

2. Immature Compost: Domestic Garden Refuse 10 Weeks (DGR 10)

The composted DGR consisted of domestic garden refuse collected from the Bisasar Road Landfill site and composted in troughs at UKZN using forced aeration technology for ten weeks conducted by Iyilade (2009, in progress). Domestic garden refuse is made up more of leaves and grass clippings from residential areas. A sample of DGR 10 can be seen in Plate 3.3.



Plate 3.3: Immature Compost: DGR 10

3. Immature Compost: Commercial Garden Refuse 10 Weeks (CGR 10)

The composted CGR consisted of the same commercial garden refuse collected from the Bisasar Road Landfill site as that of the fresh substrate as described previously. The commercial garden refuse is mainly woody consisting of branches and plant trimmings from parks and green municipal areas. The material was also composted in troughs at UKZN using forced aeration technology for ten weeks conducted by Iyilade (2009, in progress). A sample of CGR 10 can be seen in Plate 3.4.



Plate 3.4: Immature Compost: CGR 10

4. Mature Compost: Dome Aeration Technology (DAT)

CGR disposed at the Bisasar Road Landfill, was composted for over 4 months in open windrows using the Dome Aeration Technology (Griffith et al., 2006; Paar, 1999a; b; Mollekopf et al., 2002) and traditional funnel windrow composting (Etti, 2008). Dome Aeration Technology (DAT) is an advanced composting process for the aerobic biological degradation of garden refuse and general waste. It is a non-reactor open windrow composting process, where input material does not need to be turned periodically. The DAT method uses the passive aeration achieved through thermally driven advection in open windrows which is caused by the temperature differences between the degrading material and the outside environment which can be seen in Figure 3.2 (Griffith et al., 2006; Paar, 1999a; b; Mollekopf et al., 2002). A sample of Mature Compost: Dome Aeration Technology (DAT) can be seen in Plate 3.5.



Plate 3.5: Mature Compost: Dome Aeration Technology (DAT)

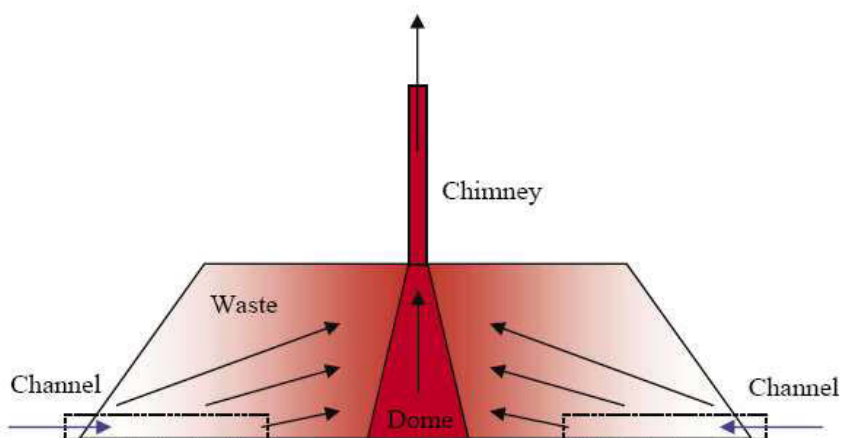


Figure 3.2: Dome Aeration Technology diagram (Griffith et al., 2006)

5. Mature Compost: Turned Windrow (TW)

The ‘turned windrow’ composting process consists of rows of long piles of organic waste known as “windrows”, that are turned on a regular basis using either manual or mechanical means, to allow for aeration to occur, causing degradation/stabilisation of the material into compost (EPA, 2009; Etti, 2008)

A sample of Mature Compost: Turned Windrow (TW) can be seen in Plate 3.6.



Plate 3.6: Mature Compost: Turned Windrow (TW)

3.2.4. Sampling

The solid substrates were sorted and sifted by hand to remove any irregular waste matter, as well as ensuring that the materials were of a relatively uniform size of approximately 4 - 5cm in length. The pine bark for example needed to be sifted in order to remove any hard wood segments.

To obtain an accurate representative sample of the solid substrates, the materials were divided into eighth fractions using the standard quartering method (Pisano, 2007). The solid substrates were mixed and turned to ensure homogeneity. The pile was then halved. These two separate halves were then mixed in turn and separated into two halves once again. This system was repeated until eight equal samples were prepared. This procedure is shown in the Figure 3.3 and Plate 3.7. Approximately 4 piles of each substrate were placed into each of the columns. The remaining samples were immediately refrigerated to prevent degradation.

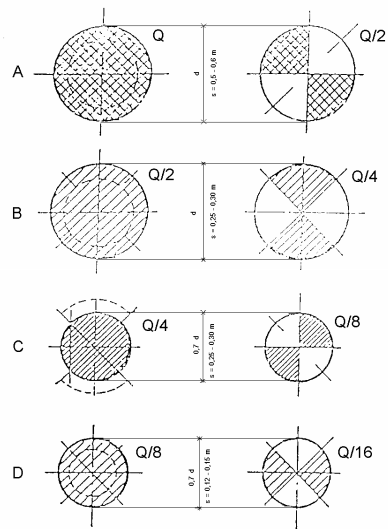


Figure 3.3: Standard quartering method (Pisano, 2007)



Plate 3.7: Quartering Method (Pisano, 2007)

3.3. Experimental Methods

3.3.1. Characterisation tests

The initial step of the experimentation was to characterise the substrates samples using conventional tests performed on the solid materials and their eluates. The eluates of the substrates were tested to determine nature as well as amounts of compounds released by the substrates whilst being in contact with water (Clesceri et al., 2005).

Solid

The solid substrate materials were tested for the following parameters:

- Moisture content (w)
- Total solids (TS)
- Volatile solids (VS)
- Respiration Index (RI₇)
- Total Carbon
- Total Nitrogen
- Carbon to nitrogen ratio (C/N)

Eluate

The eluates of the substrates were tested for the following parameters:

- Total solids (TS)
- Volatile solids (VS)
- pH
- Conductivity
- Chemical Oxygen Demand (COD)
- Biochemical Oxygen Demand (BOD₅)
- Ammonia (NH₃)
- Nitrates (NO_x)
- Total Carbon
- Total Nitrogen
- Carbon to nitrogen ratio (C/N)

A summary of the research framework for the characterisation tests can be seen in Figure 3.4.

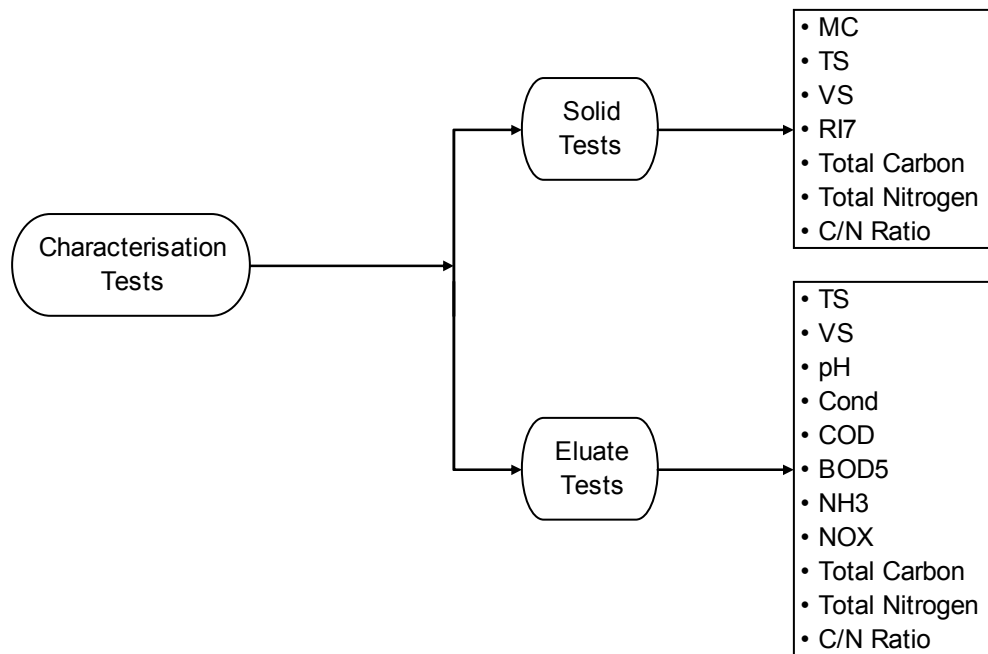


Figure 3.4: Research framework – Characterisation tests

The eluates were prepared by mixing a representative sample of each of the substrates with distilled water at a liquid to solid ratio of 10:1. These samples were then placed on a shaker for 24 hours. The samples were then filtered through a 63 micron sieve to obtain the eluate. (EN 12457-2:2003).

Tests on Solid Matter

3.3.1.1. Moisture Content (w)

The moisture content is defined as the ratio of the mass of water to the total mass of a porous medium (Bedient et al., 1999). The moisture content of the six different solid substrates was determined.

A measured amount of each substrate at natural moisture content was weighed out in six separate containers. The mass of the substrates varied from 200-900 g. The containers of substrate were then placed in an oven for 24 hrs at a temperature of 105°C for desiccation. The oven can be seen in Plate 3.8. After cooling the mass of each sample was once again measured.

The moisture content was calculated using the following equation:

$$W_{total} = \frac{w_w - w_d}{w_w}$$

Where:

w_w = wet sample mass (grams)

w_d = dried sample mass (grams)



Plate 3.8: Oven

3.3.1.2. Total Solids (TS)

(Standard Methods no. 2540 G, D, Clesceri et al., 2005)

This method is used to determine the total solids in a solid or semisolid sample. This parameter is measured by evaporating a sample to dryness and weighing the residue. The total quantity of residue is expressed in terms of a percentage on the mass of the wet sample of solid.

The test was conducted as follows:

Clean, empty crucibles were weighed. A mass of sample was then placed in each crucible. The crucibles were placed in an oven and heated at 105°C for 24 hours to evaporate the liquid leaving a residue. The crucibles were allowed to cool in a desiccator. The crucibles and dessicator are shown in Plate 3.9. The crucible was weighed again after drying to determine the mass of the dried residue. The total solids were calculated using the following equation:

$$TS (\%) = \frac{(A - B)}{(C - B)} \times 100$$

Where:

A = mass of dried residue + dish (grams)

B = mass of dish (grams)

C = mass of wet sample + dish (grams)



Plate 3.9: Crucibles and Dessicator

3.3.1.3. Volatile Solids (VS)

(Standard Methods no. 2540 G, Clesceri et al., 2005)

The fixed and volatile solids are expressed as a percentage of the dry mass/residue. The volatile solids can be determined by firing the residues from the total solids test in a furnace at approximately 550°C for about 20 minutes until the residue is converted into ash. The furnace is shown in Plate 3.10. The crucibles were then once again weighed to determine the mass of the non-volatile fixed residue after incineration.

The fixed solids are then determined using the following equation:

$$FS (\%) = \frac{(D - B)}{(A - B)} \times 100$$

The volatile solids are determined using the following equation:

$$VS (\%) = \frac{(A - D)}{(A - B)} \times 100$$

Where:

A = mass of dried residue + dish (grams)

B = mass of dish (grams)

C = mass of wet sample + dish (grams)

D = mass of residue + dish after ignition (grams)



Plate 3.10: Furnace

3.3.1.4. Respiration Index (RI₇)

The Respirometric Index at 7 days (RI₇) was used to evaluate the biodegradability of each of the substrates and their level of stability (Gomez, 2006; Adani, 2006; Adani, 2001). A respirometric system type OxiTop® was used to determine the Respiration Index at 7 days (RI₇) using the following procedure. The test was performed by adding five drops of allylthiourea to 25 g of solid material and distilled water to achieve field capacity in an airtight 1500 ml vessel. Five drops of potassium hydroxide was then added to a rubber thimble before an electronic pressure sensor head was screwed on. As biodegradation of the material occurs, oxygen is consumed and carbon dioxide produced.

The added potassium hydroxide added in the head of the vessel along with Allythiourea (ATH) absorbs the carbon dioxide (CO_2) to prevent nitrification. These can be seen in Plate 3.13. The apparatus was then placed in an incubator at 20°C for seven days. The apparatus and incubator can be seen in Figures 3.11 and 3.12. The Oxitop bottles equipped with a pressure sensor lid records the gas pressure developed during the biodegradation process of the organic matter.

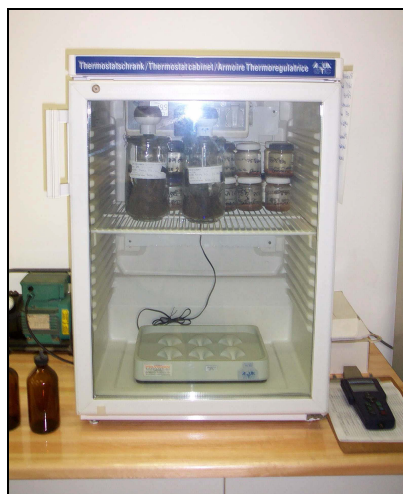
Plate 3.11: RI₇ Apparatus

Plate 3.12: Incubator

Readings of pressure were taken by an electronic handset, set at a range of 2000 mg/ℓ. These pressure readings were then used to determine the mass of oxygen consumed. The electronic handset used can be seen in Plate 3.14.

Plate 3.13: RI₇ Chemicals and Oxitop HeadPlate 3.14: RI₇ Electronic Handset

The negative pressure measured by the pressure sensor and the amount of carbon dioxide absorbed by the potassium hydroxide which is, hence, equal to the amount of oxygen consumed in the biodegradation process is directly proportional.

At the start of the test, the standard atmospheric gas composition in the vessel is assumed as in Table 3.4.

Table 3.4: Standard atmospheric air composition ratio by volume as dry air (http://www.engineeringtoolbox.com/air-composition-d_212.html accessed 15/12/2009)

Oxygen (O ₂)	Nitrogen (N ₂)	Carbon Dioxide (CO ₂)
20.95%	78.09%	0.03%

The partial pressures of Nitrogen (P_{pN}) and Oxygen (P_{pO}) are measured as follows:

$$P_{pN} = 101.3 \text{ kPa} \cdot 0.78$$

$$P_{pO} = 101.3 \text{ kPa} \cdot 0.21$$

The small amount of CO₂ absorbed is ignored. Using the Perfect Gas Law $PV = nRT$, the number of the moles is calculated for the oxygen and nitrogen moles at the start of the test. As seen in the following equation:

$$n_{O_2} = \frac{P_{O_2} V}{RT}$$

As Nitrogen is an inert gas the number of moles of nitrogen (n_N) does not change throughout biodegradation reaction. Thus the change in pressure recorded by the pressure sensor lid is used to calculate the moles of oxygen (n_{o_f}) at the end of the test. This, in turn, is used to determine the mg of oxygen consumed using the molecular weight. However, the RI₇ is measured in terms of the mass of oxygen consumed in relation to the mass of the dry material. Thus using the moisture content of the substrate, the dry mass is calculated and the RI₇ is expressed in mg_{O₂}/g_{dry mass}.

3.3.1.5. Total Carbon, Total Nitrogen and Carbon – Nitrogen Ratio (C/N)

The Total Carbon, Total Nitrogen and C/N ratio tests were carried out by BemLab in the Western Cape. The total percentage nitrogen was determined using a nitrogen analyzer following methods as laid out in the “Determination Total Nitrogen in Plant Tissue; Handbook of reference methods for plant analysis” (Horneck et al., 1998). The total percentage carbon was ascertained using the Walkley-Black method for the determination of organic carbon as stated in “Organic and humic matter, Soil and plant analysis Council, Soil analysis handbook of reference methods” (BemLab, 2009).

Eluate Tests

3.3.1.6. Total Solids (TS)

(Standard Methods no. 2540 B, D, Clesceri et al., 2005)

Solids refer to the matter suspended or dissolved in water or wastewater (Clesceri et al., 2005). The total solid test quantifies all the solids in the substance, suspended and dissolved, organic and inorganic (Tchobanoglous et al., 1985). This parameter is measured by evaporating a sample to dryness and weighing the residue. The total quantity of residue is expressed in terms of grams per litre (g/l) on a basis of the dry mass of solids.

The test was conducted as follows:

Clean, empty crucibles were weighed. 25 ml of sample were then placed in each crucible. The crucibles were placed in an oven and heated at 105°C for 24 hours to evaporate the liquid leaving a residue of the total solids. The oven is shown in Plate 3.8. The crucibles are allowed to cool in a desiccator. The crucibles and dessicator are shown in Plate 3.9. The crucibles were weighed again after drying to determine the mass of the dried residue. The total solids were calculated using the following equation:

$$TS (g / l) = W_d \frac{1000}{V_s}$$

Where:

W_d = dry mass of residue (grams)

= (mass of residue + dish before ignition) – (mass of dish)

V_s = volume of sample (ml)

1000 = multiple to convert the concentrations to g/l

3.3.1.7. Volatile Solids (VS)

(Standard Methods no. 2540 E, Clesceri et al., 2005)

The volatile solids are usually the organic content represent both the total and suspended solids (Clesceri et al., 2005). The volatile solids can be determined by firing the residues from the total solids test in a furnace at approximately 550°C for about 20 minutes until the residue is converted into ash. The furnace is shown in Plate 3.10. The crucibles were then once again weighed to determine the mass of the non-volatile fixed residue after incineration.

The non-volatile fixed solids are calculated using the following equation:

$$FS (g / \ell) = W_{FS} \frac{1000}{V_s}$$

Thus the volatile solids are determined using the following equation:

$$VS (g / \ell) = TS - FS$$

$$VS (g / \ell) = W_{VS} \frac{1000}{V_s}$$

Where:

W_{FS} = mass of the fixed residue (ashes) remaining after firing (grams)

= (mass of residue + dish after ignition) – (mass of dish)

W_{VS} = mass of the volatile residue (ashes) remaining after firing (grams)

= (mass of residue + dish before ignition) – (mass of residue + dish after ignition)

V_s = volume of sample (mℓ)

FS = concentration of non-volatile fixed solids (g/ℓ)

1000 = multiple to convert the concentrations to g/ℓ

3.3.1.8. pH

(Standard Methods no. 4500-H⁺ B, Clesceri et al., 2005)

pH is one of the most important parameters tested as a low pH is a limiting factor and has an inhibitory effect on denitrification (Trois et al., 2007; Gomez et al., 2000). The pH value of a substance is the hydrogen ion concentration. The pH is used to express the intensity of acidity or alkalinity of a solution. A pH of 7 is considered to be neutral. The basic principle of the electrometric pH measurement is the determination of the activity of the hydrogen ions. The pH of the various substances was measured using a Labotec Orion 410A pH meter. This can be seen in Plate 3.15. The pH was determined by dipping the electrode into the sample. Prior to testing the electrode had is calibrated using buffer solutions of a pH of 4 and 7.



Plate 3.15: pH meter and electrode

3.3.1.9. Conductivity

(Standard Methods no. 2510 B, Clesceri et al., 2005)

Conductivity is a measure of the ability of an aqueous solution to allow the passage of an electric current. This ability depends upon the presence of ions, their concentration, mobility, valence and the temperature at which the measurement is conducted (Clesceri et al., 2005). Conductivity in water is affected by the presence of inorganic dissolved solids. The conductivity of a solution is the measure of the ionic concentration or the amount of dissolved ions and total dissolved solids. The basic unit of measurement of conductivity is the mho or siemens. Conductivity was measured in microsiemens per centimetre ($\mu\text{S}/\text{cm}$) and millisiemens per centimetre (mS/cm). The conductivity tests were performed using a Corning conductivity meter as shown in Plate 3.16 and 3.17.

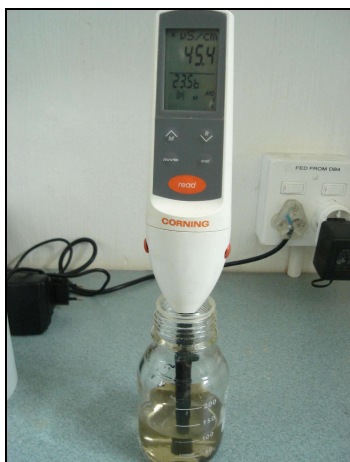


Plate 3.16: Conductivity meter



Plate 3.17: Conductivity electrode

3.3.1.10. Chemical Oxygen Demand (COD)

(Standard Methods no. 5220 D, Clesceri et al., 2005)

Chemical oxygen demand is defined as the amount of a specified oxidant that reacts with a sample under controlled conditions (Clesceri et al., 2005). The chemical oxygen demand is used to characterise the organic strength of wastewater. The test measures the amount of oxygen required for chemical oxidation of organic matter in the sample to carbon dioxide and water (Hammer, 2008). The COD test followed the procedure of the ASTM standard method. This entailed the use of the closed reflux colorimetric method.

A known sample of effluent was combined with a 1.5 mL solution of potassium dichromate ($K_2Cr_2O_7$), which is a strong oxidant, and 3.5 mL of sulphuric acid (H_2SO_4) in vials. 3 standard as well as 4 blank samples were used. The vials were placed in a digester block (Plate 3.20) for two hours at 180°C and then left to cool. A spectrophotometer (Plate 3.21), set to a wavelength of 600 nm was then used to measure the remaining dichromate in each sample.

The consumption of the oxidant is expressed in terms of oxygen equivalent using the following equation:

$$COD(mg_{O_2} / \ell) = \frac{(A - B).a}{V}$$

Where:

A = absorbance of the sample

B = absorbance of the blank sample

a = conversion coefficient (6189)

V = volume of sample

The preparation of the COD test as well as the vials of samples prior to digesting can be seen in Plates 3.18 and 3.19.



Plate 3.18: Preparation of COD test



Plate 3.19: Vials of samples



Plate 3.20: Digester block

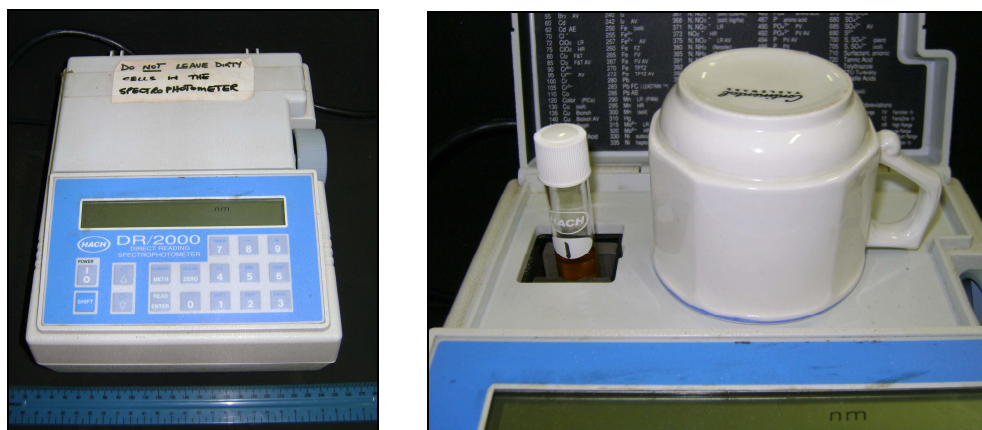


Plate 3.21: Spectrophotometer

3.3.1.11. Biochemical Oxygen Demand (BOD)

(Aqualytic, Application Report AL 99005, Robertz; Clesceri, 2005)

The biochemical oxygen demand is the amount of oxygen consumed during microbial utilisation of organics (Tchobanoglous et al., 1985). It is an important parameter used to define the biodegradable organic strength of a wastewater. The BOD is measured by placing a sample of effluent in an air-tight container which is then kept in a controlled environment, in this case an incubator for a pre-selected period of time, thus determining the amount of oxygen which is consumed. BOD is usually measured over a period of 5 days (BOD_5) and is expressed in mg/l of oxygen. The samples are placed in amber bottles (Plate 3.22) to prevent light from penetrating the sample and thus causing algae growth. The standard method for BOD testing was followed using the Aqualytic Application Report AI 99005, "Determining the Biochemical Oxygen Demand (BOD) with BSB/ BOD Sensors, manometric method" (Aqualytic, Application Report AL 99005, Robertz).



Plate 3.22: BOD Apparatus

3.3.1.12. Ammonia (NH_3)

(Standard Methods no. 4500 – NH_3 B, C, Clesceri et al., 2005)

Ammonia nitrogen ($\text{NH}_3\text{-N}$) is present in its aqueous form (NH_4OH) as well as an ion of ammonia (NH_4^+) depending on the pH. Initially the use of the distillation and titration procedure was followed however due to problems experienced with the equipment the samples were sent to Stewart Inspection and Analysis a private laboratory.

The initial distillation method used involved distilling 50 mL of sample which was placed in a glass flask, into a solution of boric acid which acts as an absorbent, as shown in Plate 3.23. Once a 250 mL solution of distilled sample and boric acid is produced, this is titrated with standard hydrochloric acid (HCl) titrant 0.01N to obtain the amount of ammonia in mg/L of Nitrogen.



Plate 3.23: Ammonia Distillation apparatus

3.3.1.13. Nitrates (NO_3)

A colorimetric method was used to determine the concentration of nitrates present. Merckoquant Nitrate test was conducted using nitrate sticks. The stick is dipped in the sample for approximately 1 second and a reading is taken after 1 minute. The colour is then compared on a range of 0 – 500 mg/l. However if nitrites were indicated on the stick, 5 drops of a 10 % aqueous amidosulfonic acid solution were used in each sample to absorb nitrites. The nitrate test, sticks and the 10 % aqueous amidosulfonic acid solution are shown in Plate 3.24 and Plate 3.25 respectively.

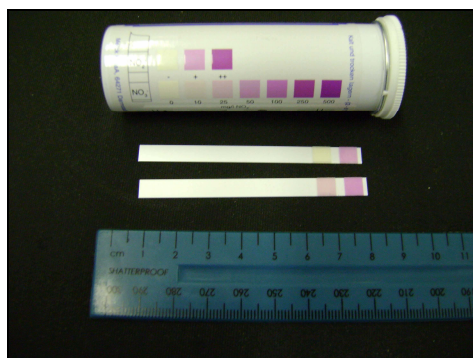
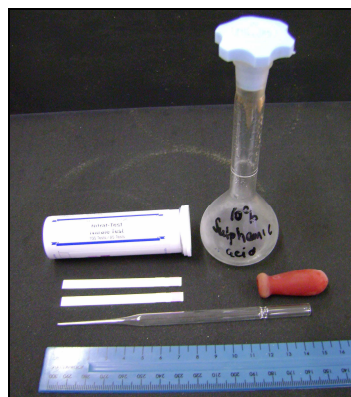


Plate 3.24: Nitrate sticks

Plate 3.25: 10 % Aqueous
Amidosulfonic acid solution

3.3.2. Batch Tests

The suitability of each of the substrates as carbon sources for denitrification was initially assessed using small scale dynamic batch tests (Tsui et al., 2007). The research framework used for the batch tests is seen in Figure 3.5.

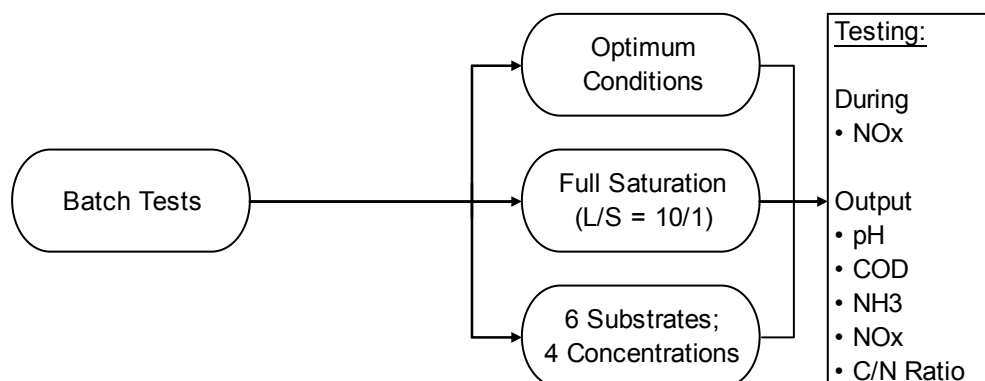


Figure 3.5: Research framework - Batch tests

The batch tests were designed to determine the kinetics of removal of each substrate at optimal conditions, which were maximum contact between the substrate and solution, a pH range between 6 to 8 and at a temperature of approx. 25°C. A Liquid to Solid ratio of 10:1 was used for all tests to ensure full saturation.

The batch tests were conducted at 3 different nitrate (NO_3^-) concentration levels: 100, 500 and 2000 mg/l, and a blank test (0 mg/l) was performed using distilled water for the same duration of the test at 500 mg/l.

All tests were conducted in duplicate or triplicate in closed top batch reactors consisting of 1 l, 3 neck bottles equipped with two airtight silicone septa which allowed continuous sampling thus preventing any ingress. The apparatus can be seen in Plate 3.26. Each bottle was filled with 100 g dry matter of substrate and respective concentration of potassium nitrate solution (KNO_3). The substrate particles were cut and reduced to a standard size of 4 – 5 cm. Prior to adding the nitrate solution, the bottles filled with substrate, were flushed with nitrogen gas to ensure anaerobic conditions.

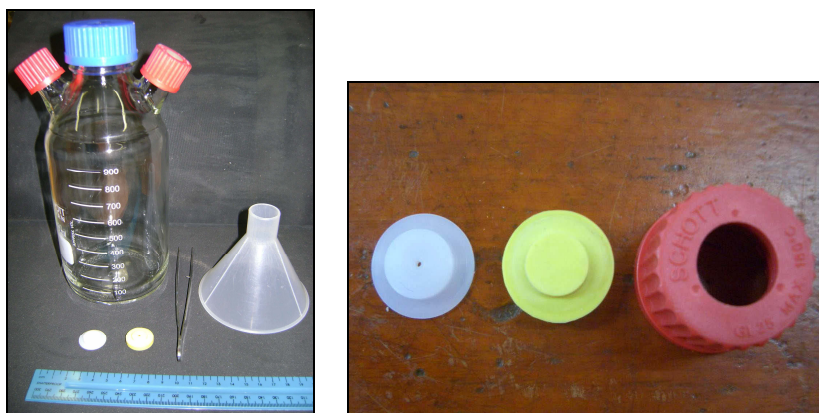


Plate 3.26: Batch test apparatus

The batch reactors were placed in a shaker at 150rpm at a controlled room temperature of approx. 25°C (Seen in Plate 3.27). Small samples of approximately 1-5 ml were extracted using a gas tight syringe so as to test the nitrate concentration (NO_3) after 5, 10, 15, 30 and 60 minutes during the first hour of testing and every hour after that for the first day, thereafter 3 times a day usually every 3 hours depending on any changes in nitrate concentration. Shown in Plate 3.28 and 3.29. This method of extraction was performed in order to not significantly affect the L/S ratio in the reactors and to ensure that full saturation was maintained. The nitrate concentrations for the batch tests were determined using the nitrate sticks (Chapter 3, Section 3.3.1.13.). In some instances, the amount of fines in the tests prevented an accurate reading on the nitrate sticks. Thus some of the samples were filtered using a 0.45 μm filter as shown in Plate 3.30.

The batch tests were conducted until the nitrate concentration reached zero. At the end of the test, the sample was sieved using a 63 micron sieve and characterised using eluate testes (Chapter 3, Section 3.3.1.).



Plate 3.27: Shaker



Plate 3.28: Batch test sampling

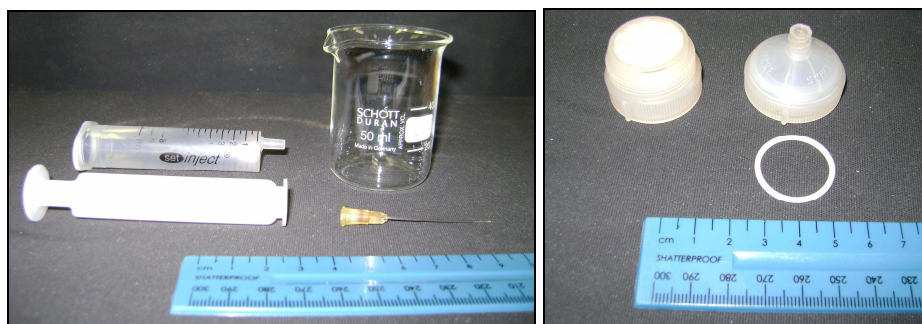


Plate 3.29: Syringe, needle and filter

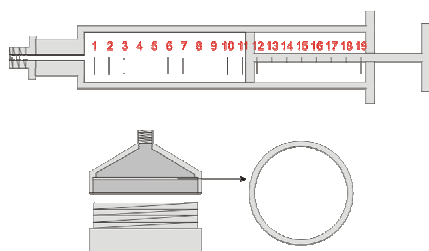


Plate 3.30: Siring 0.45 μ m filter and schematic view

3.3.3. Column Studies

Three substrates were selected for the large scale experiments in columns due to their biodegradability and availability of carbon in particular reference to the carbon to nitrogen ratio, Respiration Index as well as their performance in terms of denitrification. Leaching columns were used to simulate the denitrification process in a fixed-bed reactor (Tsui et al., 2007; Gomez et al., 2000; Volokita et al., 1995). The research framework used for the columns studies are shown in Figure 3.6.

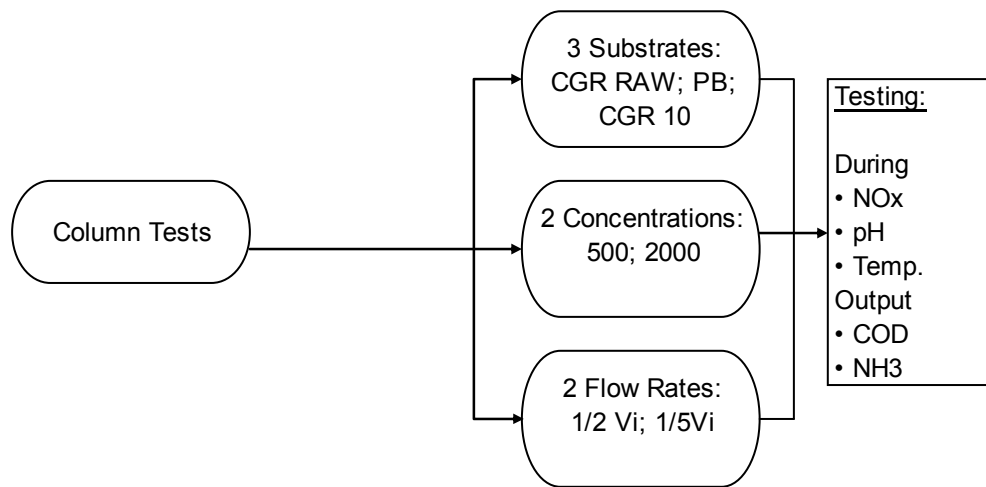


Figure 3.6: Research framework - Column studies

Two different experiments were conducted using the columns to investigate the effect of denitrification rates for different nitrate concentration levels and flow rates. These results were used to determine the kinetics of removal, loading rates and hydraulic retention time for the filter beds.

Three substrates were then selected for the large scale experiments in columns due to their biodegradability and availability of carbon in particular reference to the carbon to nitrogen ratio, Respiration Index as well as their performance in terms of denitrification. The substrates chosen were the fresh pine bark (PB), the fresh CGR and the immature CGR compost.

Two nitrate concentrations (500 and 2000 mg/ℓ) and two different flow rates as seen in Table 3.5, were used for the column campaign. These concentrations were chosen as a result of the typical ranges of nitrate concentrations displayed by the treated landfill leachate produced by the Sequencing Batch Reactor (SBR) at the Mariannhill Landfill site as seen in Tables 3.2 and 3.3 in Section 3.2.1.

It has been established that flow through a reactor improves the efficiency of denitrification on the postulation that water circulation favoured organic matter release and dispersion (Tsui et al., 2007; Diaz et al., 2003; Volokita et al., 1995). However a flow rate that is too high may cause a drop in the rate of removal (Volokita et al., 1995). Two different flow rates were thus chosen to ascertain the limiting flows and thus retention time that effect denitrification. The first two flow rates were applied for a period of over 4 weeks. The second experiment was prolonged to ascertain the affect the previous flow rates had on the substrates. The columns were thus left in flooded conditions for a period of 1 week.

3.3.3.1. Equipment

The columns were constructed using a transparent PVC cylindrical body, plastic flanges with valves, rubber gaskets (seals) and stainless steel bolts.

Characteristics of the columns:

The transparent PVC cylindrical body was 1 m in length, 160 mm in diameter and had an approximate volume of 20 litres. Three ports were also installed along the length of the columns to allow sampling to occur throughout the length. A Perspex diffuser was made and fitted in the top of each column to ensure that the solution was distributed throughout the entire girth.



Plate 3.31: Leaching Columns

The upper and lower ends of the columns were closed using two pairs of 25 mm thick and 280 mm in diameter plastic flanges. A 20 mm rubber gasket was placed between each of the flanges using a silicon gel to ensure an airtight fit. The other end of each of the flanges were then bolted together using stainless steel bolts. The column was then bolted to a steel frame.



Plate 3.32: Plastic flanges

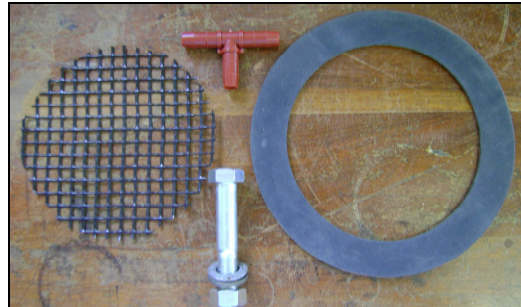


Plate 3.33: Rubber gasket, filter and bolt

The upper flange consisted of two orifices. The first is a tap valve which allows the nitrate solution to be poured into the column. The second is connected to a small plastic pipe which is used to measure the biogas production. The lower flange has only the outlet orifice. This tap valve is connected to a pipe which allows the column to be drained and the effluent collected. These can be seen in Plate 3.34.

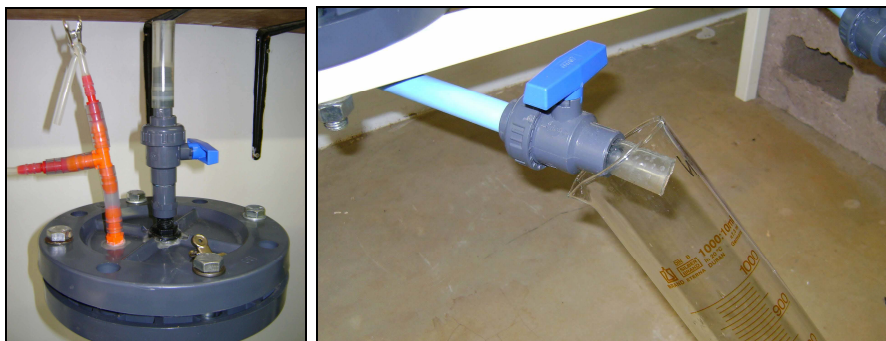


Plate 3.34: Upper flange and lower flange with tap valve

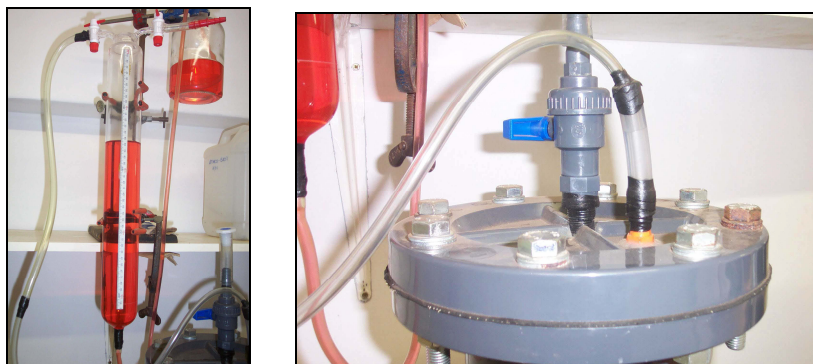


Plate 3.35: Upper flange with biogas collection system

A coarse filter and a layer of marbles were placed at the bottom of each column to provide a drainage layer, thus preventing any substrate from obstructing the outlet. The operating conditions presented in Table 3.5.

Table 3.5: Summary of column operating conditions

Column	Substrate	NO ₃ Concentration (mg/l)	Duration (Weeks)	Flow Rates (l/day)	
				Experiment 1	Experiment 2
1	CGR RAW	500	4	2.48	5.625
2	PB	500	4	2.00	5.00
3	CGR 10	500	4	1.70	2.85
4	CGR RAW	2000	4	2.38	5.65
5	PB	2000	4	2.00	5.00
6	CGR 10	2000	4	1.78	2.85

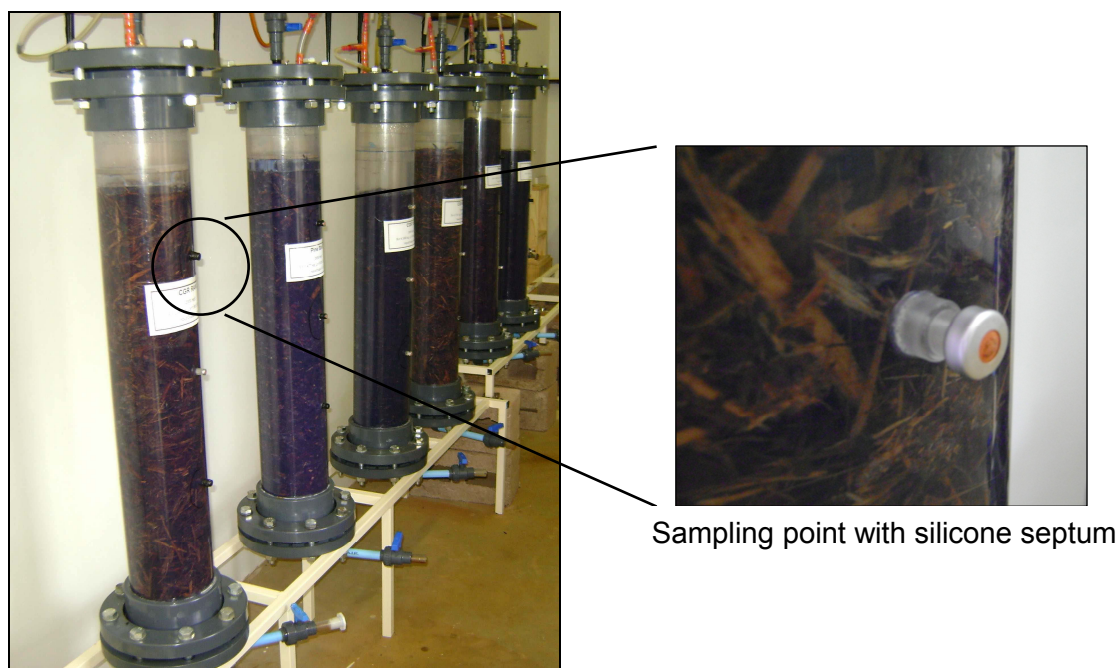


Plate 3.36: All 6 Columns with sampling points

The first three columns were filled with a 2000 mg/l synthetic nitrate solution and the second three columns were filled with a 500 mg/l synthetic nitrate solution. Enough solution was added to each column to ensure that the substrates were covered. The initial input conditions are shown in Table 3.6 and 3.7.

Table 3.6: Initial input conditions of each column (2000 mg/ℓ)

Column Input (2000 mg/ℓ)	CGR RAW (kg)	PB (kg)	CGR 10 (kg)
Total input mass	2.800	3.477	6.386
Moisture Input	1.040	1.698	4.280
Dry Mass	1.760	1.779	2.106
Added Nitrate Solution	11.900	10.000	8.900
Total Moisture	12.940	11.698	13.180
L/S Ratio	7.35	6.58	6.26

Table 3.7: Initial input conditions of each column (500 mg/ℓ)

Column Input (500 mg/ℓ)	CGR RAW (kg)	PB (kg)	CGR 10 (kg)
Total input mass	2.731	3.422	6.566
Moisture Input	1.014	1.672	4.401
Dry Mass	1.717	1.750	2.165
Added Nitrate Solution	12.400	10.000	8.500
Total Moisture	13.414	11.672	12.901
L/S Ratio	7.81	6.67	5.96

Total input mass = Moisture Input + Dry Mass

Total moisture = Moisture Input + Added Nitrate Solution

L/S Ratio = Total Moisture/ Dry Mass

When draining the columns, nitrogen gas was pumped into each of the columns to ensure that the experiment was kept anaerobic.

3.3.3.2. Experiment 1

For the initial experiment the columns were filled with a 500 mg/ℓ and 2000 mg/ℓ nitrate solution respectively. The experiment was designed to assess the nitrate removal capabilities of the substrates at a relatively low flow rate.

It was decided that the entire volume of nitrate solution should be replaced over a 5 day period. Thus 1/5 of the initial input volume of nitrate solution was sampled from the bottom of the column everyday and replaced with the nitrate solution. The first litre of effluent was discarded as it would not have been in contact with the substrate but rather with the marble filter. The effluents were analysed for NO₃, DO, pH and temperature daily and for COD and NH₃ once a week. This test was run for a 4 weeks.

3.3.3.3. Experiment 2

This experiment was performed to investigate the nitrate removal capabilities of the columns at a high flow rate. The columns were thus drained of their effluent and filled with the same concentrations of nitrate solution as used in Experiment 1 until the substrates were covered.

It was decided that the entire volume of nitrate solution should be replaced over a 2 day period. Thus 1/2 of the initial input volume of nitrate solution was sampled from the bottom of the column everyday and replaced with the nitrate solution.

Once again the first litre of effluent was discarded as explained in Experiment 1. As in Experiment 1, effluents were analysed for NO_3 , pH and temperature daily and for COD and NH_3 once a week. The DO test was not used in this experiment as accurate readings could not be obtained due to the turbulent flow at which the effluent was collected from the columns. This test was run for a 4 weeks. The test was prolonged to ascertain the affect the previous flow rates had had on the substrates. The columns were thus left in flooded conditions for a period of 1 week. The nitrate levels were tested every day.

3.3.4. Durability Tests

Durability testing was done on 4 columns that had been used in previous studies to determine whether the substrates still had denitrification capabilities over an extended period of time.

The Research framework followed for the Durability tests is shown in Figure 3.7.

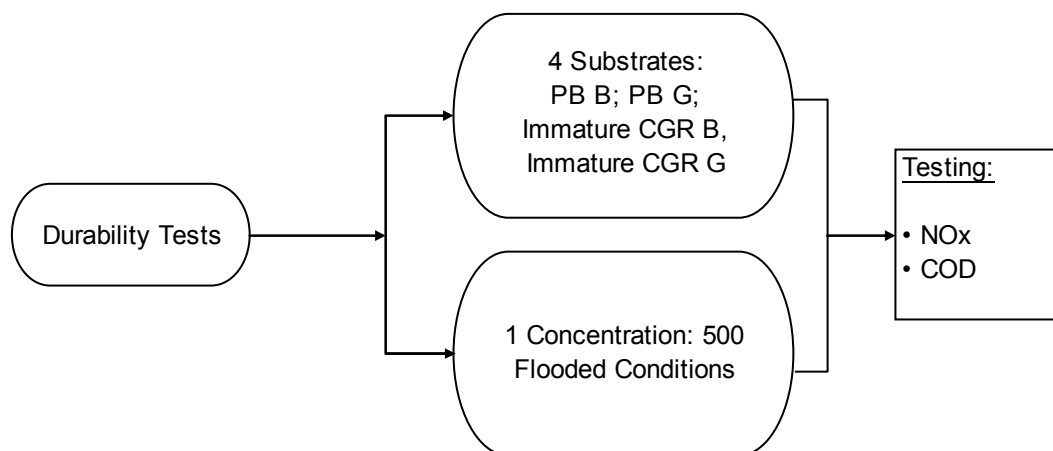


Figure 3.7: Research framework - Durability test

The 4 columns were filled with Pine Bark and Immature Compost. Both pairs were in operation for almost a year and 2 years in the following condition:

The columns were initially drained and refilled with a 500 mg/l nitrate solution until the substrates were entirely covered. The columns were run in flooded conditions. Once a day a sample was collected from the bottom of the column and replaced with the nitrate solution. As discussed in Section 3.3.3.2., the first litre of effluent was discarded as it would not have been in contact with the substrate but rather with the marble filter. The effluents were analysed for NO₃ daily and for COD three times per cycle. A cycle was the period in which each column took to reach a zero nitrate concentration. Once each column had reached this point, the column was drained and refilled. This experiment was conducted for approximately 7 months.

The columns were called Pine Bark and Immature Compost Björn (PB – B and IC – B) and Pine Bark and Immature Compost Giulia (PB – G and IC – G).

3.3.5. Biogas Analysis

All the columns were equipped with a biogas measurement system based on the liquid gas displacement method. The system is connected to the top flange of the column as shown in Plate 3.37. The system comprises of a 2 litre glass bottle which is used as a reservoir and a 1.2 litre graduated glass burette with two taps. The one tap is connected to the top of the column via a plastic pipe and the other tap is used for the gas analyser.

The burette is filled with a liquid solution of sodium chloride (NaCl), sulphuric acid (H_2SO_4) and a red colorant.

The biogas is produced, flows through the plastic pipe and into the burette, thus causing the liquid to be displaced. The volume of biogas produced is thus equivalent to the volume of solution displaced. This volume is thus measured in the graduated burette.

A gas analyser type GA2000 as shown in Plate 3.38, was used to determine the percentage of methane (CH_4), carbon dioxide (CO_2) and oxygen (O_2) produced to ensure that the columns did not become methanogenic, which would be noticed in terms of excessive CH_4 production. The gas analyser pumps the air out of the burette and thus the displaced liquid is levelled with that in the reservoir at atmospheric pressure. Thus the levels are zeroed after each measurement.

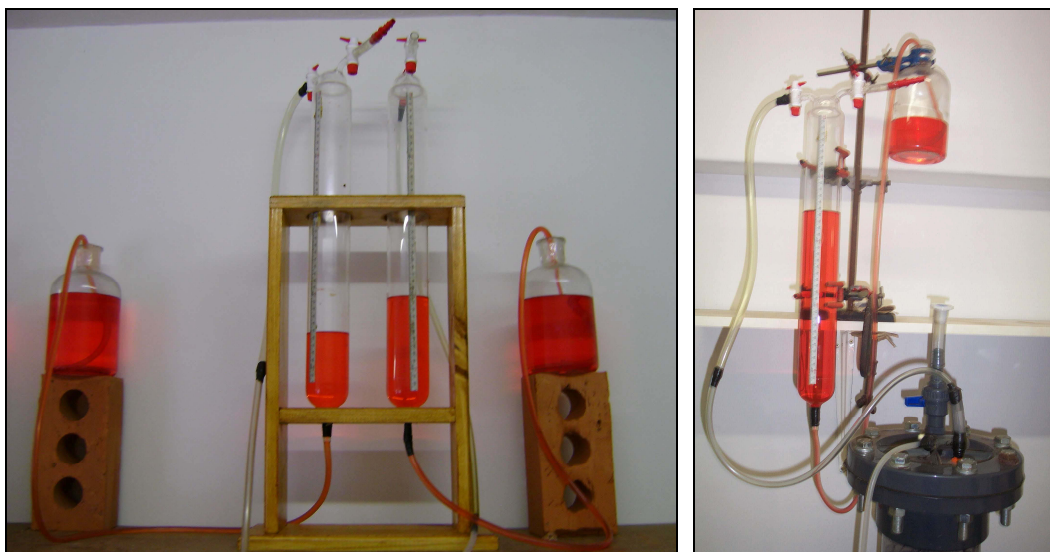


Plate 3.37: Biogas system of columns



Plate 3.38: Gas Analyser type GA2000

During all the column experiments, nitrogen gas was pumped into the columns during drainage to ensure anaerobic conditions were maintained. Thus during drainage and refilling the biogas measurement equipment was isolated.

3.3.6. Microbial Analysis (De Combret, 2009)

Microbial analyses were conducted in parallel with this study as part of De Combret, 2009, on batch tests at a nitrate concentration of 500 mg/l for immaturesly composted commercial and domestic garden refuse (CGR 10 and DGR 10) and pine bark (PB).

A semi-quantitative analysis of the effect of the substrates on the growth of the bacterial communities was conducted following the 9215-C spread plate method (Clesceri et al., 2005). Small samples drawn from the batch tests were diluted with a sodium chloride solution and spread on a 90 mm agar plate using Luria-Bertani Broth. The plates were incubated at room temperature (approx. 25°C) in the dark and the aerobic cultivable microflora was enumerated visually after 3 days (De Combret, 2009; Trois et al., 2010). The colonisation of the substrates was assessed using microscopic analysis through an Environmental Scanning Electronic Microscopy (ESEM).

A phylogenetic analysis of the bacterial population to provide an insight into the composition of the bacterial community. The effect of an inoculum was also studied. Findings of the work can be seen in Appendix E.

In the next chapter, the results achieved under various experiments will be presented and discussed.

CHAPTER 4

4. RESULTS AND DISCUSSION

4.1 Introduction

The initial section of this chapter presents and discusses the results of the substrates' characterisation tests. The batch tests are then described firstly in terms of the fresh substrates (CGR RAW and PB), followed by the immaturely composted substrates (CGR 10 and DGR 10) and finally by the mature substrates (DAT and TW). This section presents a characterisation of the input and output material, nitrate concentrations with time and kinetics and percentage of nitrate removal.

The final section presents the results of the column studies beginning with the selection of the substrates, followed by a direct comparison of their performance for two different flow rates and nitrate concentrations, and ending with durability tests. This chapter includes discussion of the evolution of the nitrate concentrations with time and along the length of the column, pH, COD and ammonia concentrations with time, and modelling of the nitrate removal kinetics and hydraulic retention time in columns.

4.2. Characterisation Results

The characterisation of both solid substrate materials and their eluates using procedures outlined in Chapter 3 are shown in Table 4.1 and 4.2. The eluates of the substrates were tested to determine the nature as well as the amounts of compounds released by the substrates whilst being in contact with distilled water. The results shown in this chapter are the average of the data obtained. The raw data of the tests can be seen in Appendix A.

Table 4.1: Characterisation of fresh substrates

	Fresh Material			
Test/Substrate	DGR RAW	CGR RAW	PB (Moist)	PB (Dry)
Solid				
MC (%)	56.90 ± 4.32	37.14 ± 3.17	48.85 ± 2.92	22.66 ± 3.90
TS (%)	43.10 ± 4.32	62.86 ± 3.17	51.15 ± 2.92	77.34 ± 3.90
VS (%)	82.06 ± 1.42	96.37 ± 0.75	97.08 ± 0.17	97.01 ± 0.81
RI7 (mg O ₂ /g DM)	16.176	7.770	17.769	8.598
Total C (%)	41.70	49.6	36.67	38.46
Total N (%)	0.75	0.55	0.59	0.43
C/N Ratio	55.60	90.19	62.15	89.44
Eluate				
TS (g/l)	20.08 ± 0.19	4.08 ± 0.02	3.66 ± 0.01	4.26 ± 0.02
VS (g/l)	12.03 ± 2.24	3.04 ± 0.02	3.35 ± 0.28	3.76 ± 0.02
pH	5.63	5.45	4.18	4.93
Cond (mS/cm)	5.21	1.653	0.845	0.847
COD (mg/l)	18412	4253	4517	4443
BOD5 (mg/l)	3345	1101	297	225
NH ₃ -N (mg/l)	178.92	12.74	8.54	9.10
NO _x -N (mg/l)	13.30	6.86	15.12	0
Total C (%)	0.48	0.083	0.25	0.39
Total N (%)	0.07	0.0183	0.07	0.05
C/N Ratio	6.86	4.54	3.57	7.80

Tables 4.1 and 4.2: The ± values refer to the standard deviation of the results. The standard deviation is only included when the test has been done in triplicate or greater.

Table 4.2: Characterisation of composted substrates

	Immature Compost		Mature Compost	
Test/Substrate	DGR 10	CGR 10	TW	DAT
Solid				
MC (%)	66.05 \pm 4.71	67.03 \pm 0.83	59.28 \pm 3.22	54.24 \pm 2.90
TS (%)	33.95 \pm 4.71	32.97 \pm 0.83	40.72 \pm 3.22	45.76 \pm 2.90
VS (%)	62.38 \pm 9.84	89.62 \pm 1.40	71.73 \pm 2.42	87.20 \pm 8.68
RI7 (mg O ₂ /g DM)	14.123	5.672	9.823	6.987
Total C (%)	23.97	28.69	29.04	22.04
Total N (%)	1.88	1.20	1.65	0.96
C/N Ratio	12.75	23.91	17.60	22.96
Eluate				
TS (g/l)	16.65 \pm 2.77	2.40 \pm 0.10	12.55 \pm 0.14	11.78 \pm 0.26
VS (g/l)	12.00 \pm 0.18	1.62 \pm 0.07	8.61 \pm 0.14	7.55 \pm 0.29
pH	7.40	6.98	7.27	6.93
Cond (mS/cm)	4.98	0.81	2.69	1.23
COD (mg/l)	17556	2764	11270	10080
BOD5 (mg/l)	350	155	474	348
NH ₃ -N (mg/l)	82.04	9.80	50.12	29.40
NO _x -N (mg/l)	15.2	7.14	14.56	8.96
Total C (%)	1.0	0.11	0.67	0.60
Total N (%)	0.12	0.06	0.09	0.07
C/N Ratio	8.30	1.83	7.44	8.57

The results in Table 4.1 and 4.2 suggest that pine bark, as well as both the fresh garden refuses (DGR RAW and CGR RAW) are both acidic. pH is a limiting factor in the denitrification process and thus the low pH values will impact negatively on the rate of nitrate removal as the optimum pH for biological denitrification is between 6 and 8. The acidic nature of especially the pine bark will cause an inhibitory effect on denitrification. As a result of degradation and the high production of NH₃, pH levels in the composted materials are closer to neutral and in some cases alkaline (Adani et al., 2006). This can be seen especially in the DGR 10 and TW values. The composting has produced favourable pH values as they now fall into of the optimum range for degradation.

The total solids determined in the eluates from the substrates, show that the raw garden refuses have a higher amount of total solids than the immaturely composted material. However, both the mature composts have a higher amount of total solids. This may be due to the composting process which mobilises the degraded fine particles increasing the TS concentration in solution.

It is also noted that there is a strong correlation between TS and COD, as higher TS levels reflect in higher percentage of total carbon in the eluates. This suggests that carbon can be easily released, mobilised by the composting process and can be easily released by leaching for denitrification.

The higher carbon content, in the form of COD and BOD for both the raw garden refuse and pine bark compared to that of the immature and mature compost is expected, as these substrates have not undergone any stabilisation. The high COD results of the pine bark and raw garden refuse are also due to the fact that the substrates are organic materials.

All organic matter has a ratio of carbon to nitrogen in its tissues which affects the course of decomposition as organisms use carbon as a source of energy to decompose this organic matter and thus need a higher carbon content than nitrogen (<http://www.gardensimply.com/compostcn.php> accessed 15/12/2009 and http://whatcom.wsu.edu/ag/compost/fundamentals/needs_carbon_nitrogen.htm accessed 15/12/2009).

The typical range for stabilised compost is between 13 – 16 (Tsui et al., 2007; Wu et al., 2002). The DAT, DGR 10 and CGR 10 fall outside this range, with DAT and CGR 10 having a greater C/N ratio. This should make these two materials appropriate for denitrification. The lower C/N ratio displayed by the composted material is due to its maturity and stability. The ideal initial C/N ratio to obtain good compost is 20 – 35 (http://whatcom.wsu.edu/ag/compost/fundamentals/needs_carbon_nitrogen.htm accessed 15/12/2009).

Grass clippings should have a typical C/N ratio of 19 where as leaves vary from 35 – 85. (http://whatcom.wsu.edu/ag/compost/fundamentals/needs_carbon_nitrogen.htm as accessed 15/12/2009). It can be seen from the determined C/N ratio of the DGR RAW substrate which consists mainly of these two materials that a C/N ratio of 55.60 is appropriate and within range.

Pine bark on the other hand, has a determined C/N ratio between 62 – 90. According to the literature researched by Trois et al. (2007) and Pisano (2007), the C/N ratio in pine bark is very high with values differing drastically from 723:1 (Willson, 1989), 580:1 (Schliemann, 1974), 480:1 (Lamb, 1982) and 300:1 prior to composting and 150:1 after

composting (Gartner, 1979). Thus the pine bark used in this research has a lower C/N ratio than that stated in the literature, but still above that of the DGR RAW substrate. The C/N ratio of the pine bark substrate is comparable to that of the fresh garden refuse materials and still has a high C/N ratio.

All the materials used have a similar composition in the fact that they have higher carbon (C) content in comparison to nitrogen (N). This characteristic makes these materials well suited for nitrate removal as they provide organic carbon for denitrification without increasing the nitrogen concentration.

The RI_7 or respiration test as proposed by Adani et al. (2001) assesses the biodegradability and biological stability of the material by determining the amount of oxygen consumed by the indigenous biomass that is present in the substrate to degrade the material. "The biological stability indicates the extent to which readily biodegradable organic matter has decomposed" (Adani et al., 2006; Gomez et al. 2006). An unstable material is considered to contain a high portion of biodegradable matter that must sustain high microbial activity (Gomez et al., 2006; Chroni et al., 2009).

As described by Gomez et al. (2006) the respiration is directly related to the metabolic activity of the microbial population. Large amounts of bioavailable organic matter cause micro-organisms to respire at a higher rate than that if the material is scarce of organic matter (Gomez et al., 2006). Respiration has become an important parameter in the composting process for ascertaining the stability of the material (Gomez et al., 2006).

As defined by Adani et al. (2006) compost is a stable, mature and humified material. The quality of compost is assessed according to both the maturity and stability parameters (Gomez et al., 2006). The respiration activity is measured as O_2 consumption and/or CO_2 production by the composting mass (Chroni et al., 2009; Gomez et al., 2006).

As expected the immaturely composted materials of DGR and CGR have lower RI_7 values than their fresh counterparts. This indicates that during the composting process the materials have not only become more mature but also more stable. The fresh raw materials thus have a high portion of biodegradable matter that must sustain high microbial activity.

What is interesting is that the composted CGR 10 substrate which has been composted using forced aeration at UKZN has a lower RI_7 value than both the maturely composted materials. This suggests that it is not only more mature but also more stable, making it higher quality compost. This indicates that the composting efficiency achieved, in the forced aeration troughs at UKZN, was relatively higher than those produced from Bisasar Road Landfill.

The high levels of ammoniacal nitrogen ($NH_3 - N$) present in both the domestic garden refuse samples is also noticed. This may cause increased nitrate levels through bioleaching. The production or leaching of NH_3 from the substrates will cause a rise in nitrogen. If there is sufficient oxygen present in either the solution or the pores of the substrate, NH_3 could be converted into NO_2 .

4.3. Batch Tests

The results of the small scale dynamic batch tests were used as indicators for the selection of substrates to be used in the larger scale column tests. The tests were conducted at optimal conditions, these being maximum contact between the substrate and solution, at pH 6-8 and at a room temperature of approx. 25°C. The raw data of the tests can be seen in Appendix B.

4.3.1. Pine Bark

The characterisation results of the tests performed on the input and output of the solid substrate and their eluates in the batch tests at the different initial nitrate concentrations are shown in Table 4.3.

Table 4.3: Characterisation results of the input and output of the Pine Bark batch tests

Parameter	Input Eluate	Input Solid	Blank (0 mg/l)		100 (mg/l)		500 (mg/l)		2000 (mg/l)	
			Output Eluate	Output Solid	Output Eluate	Output Solid	Output Eluate	Output Solid	Output Eluate	Output Solid
pH	4.18		4.90		5.10		4.30		4.64	
COD (mg/l)	4517		11192		5021		14157		13245	
NH ₃ -N (mg/l)	8.54		3.5		2.25		22.5		30	
NO ₃ (mg/l)	15.12		0		0		255		1600	
Total C (%)	0.25	36.67		52.4		48.5		52.0		48.9
Total N (%)	0.07	0.59		0.61		0.66		0.59		0.29
C/N Ratio	3.57	62.15		85.9		73.57		88.81		343.26

The pH throughout all the batch tests stayed acidic, ranging from 4.30 to 5.10. The nitrate concentration (NO₃) reached zero only in the case of the test at 100 mg/l. The other two tests failed to reach full denitrification.

There was a presence of positive bioleaching of carbon which was observed in the increase of both the COD and C/N ratios, relating to the initial nitrate concentration. The COD results showed an increase from the initial input ranging from 5021 – 14157 mg/l. There was also an increase in NH₃ which correlates to the reduction in total N (%) from 0.59 – 0.29, which indicated there was also bioleaching of nitrogen. The increase in COD was greater than that experienced in NH₃ resulting in an increased C/N ratio. As C/N ratio was calculated using wet samples, carbon leached out from the substrate was still trapped in the biofilm of the pores resulting in the observed increase in C/N Ratio from 62.15 to 343.26.

The evolution of the nitrate concentrations for the Pine Bark substrate conducted for each of the concentrations are shown in Figures 4.1, 4.2 and 4.3. The graphs demonstrate the nitrate concentration (NO_3) in mg/ℓ in relation to time in days. Due to the small variety in the blank test, its results are included in each of the graphs.

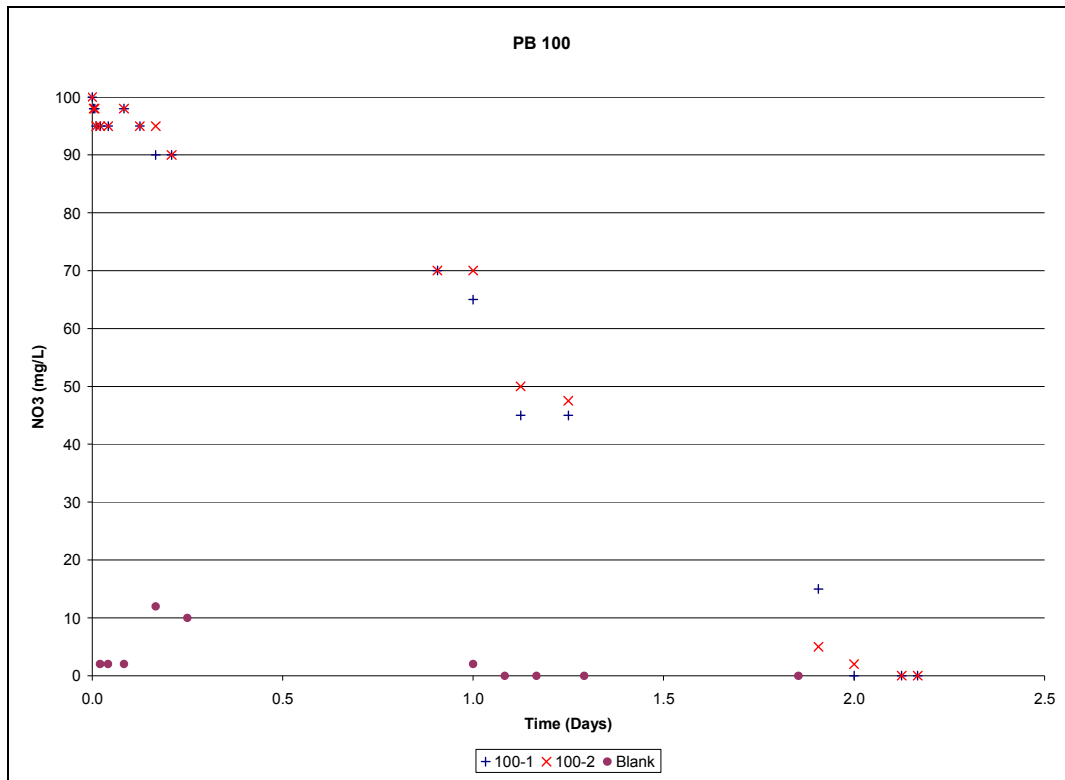
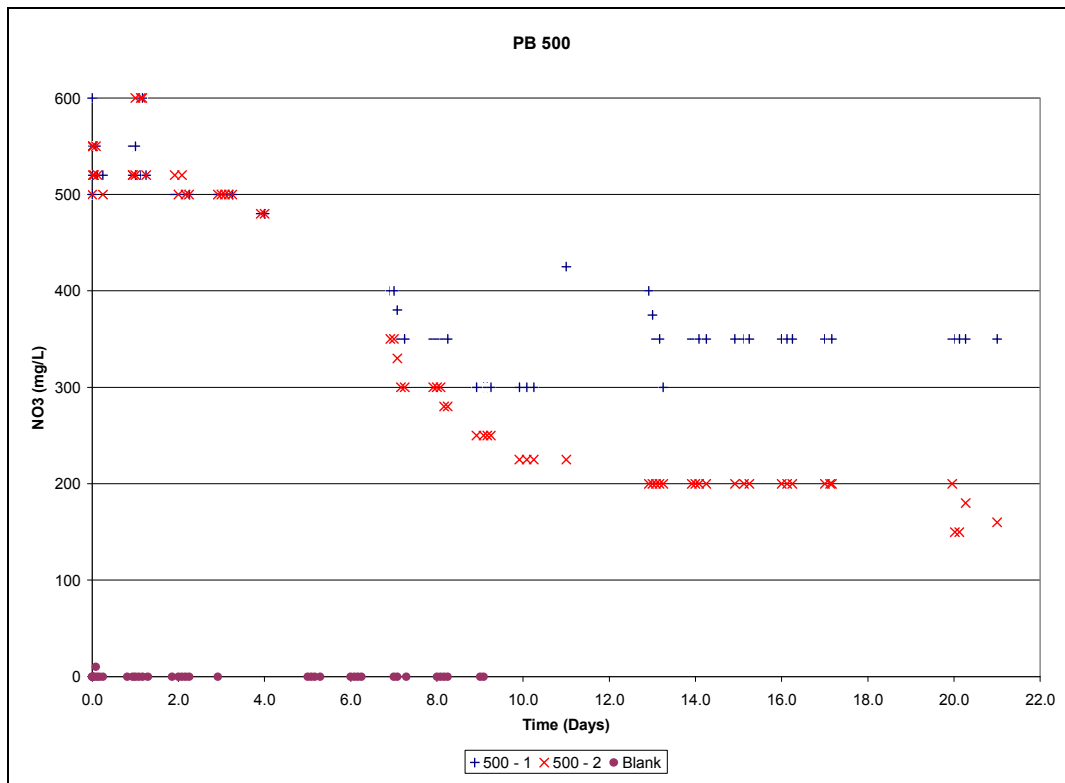
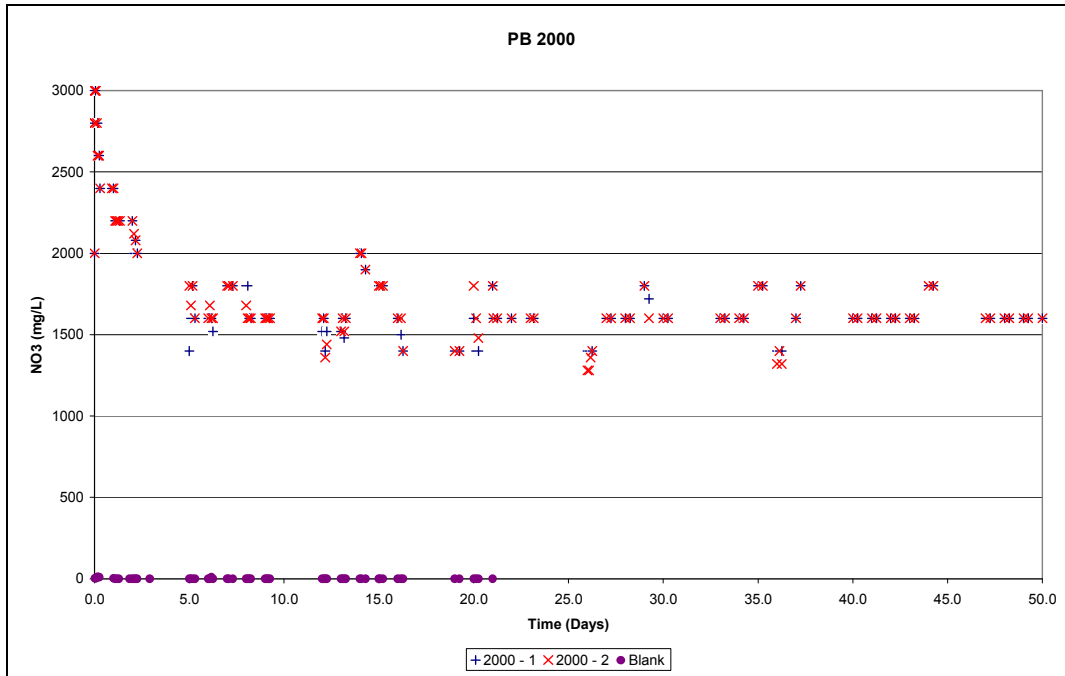


Figure 4.1: Evolution of the nitrate concentration for Pine Bark at $C_0 = 100 \text{ mg}/\ell$

Figure 4.2: Evolution of the nitrate concentration for Pine Bark at $C_0 = 500 \text{ mg/ℓ}$ Figure 4.3: Evolution of the nitrate concentration for Pine Bark at $C_0 = 2000 \text{ mg/ℓ}$

Kinetics: Rate of Reaction

A zero order model was applied to the given results. $\frac{dc}{dt} = -k \rightarrow c = c_o - kt$

Rate of Reaction for linear period:

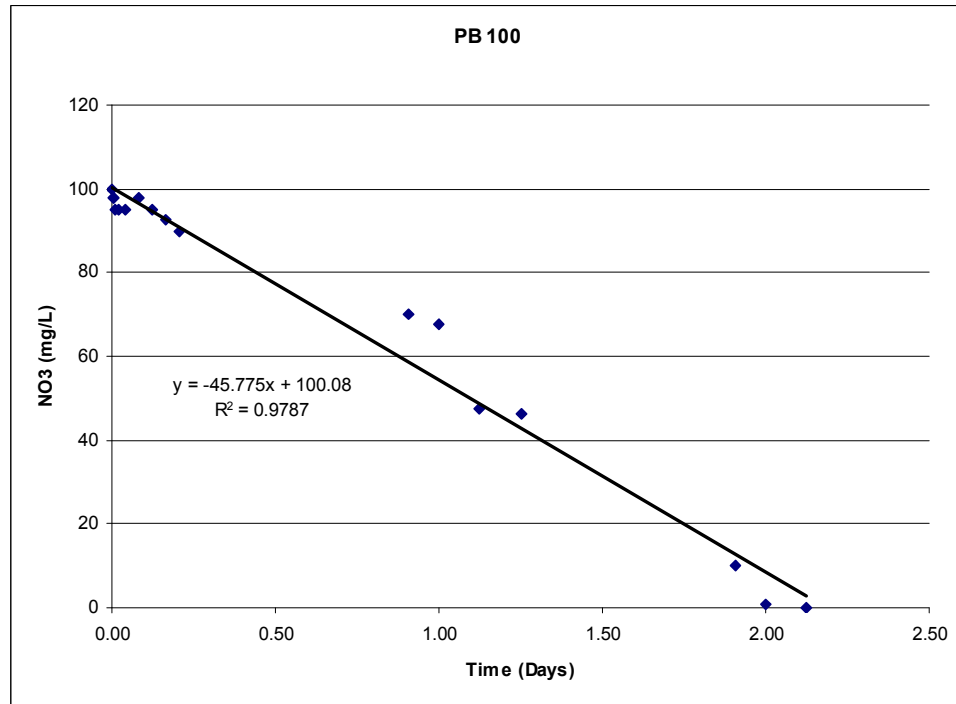


Figure 4.4: Kinetics of PB at $C_o = 100 \text{ mg/}\ell$

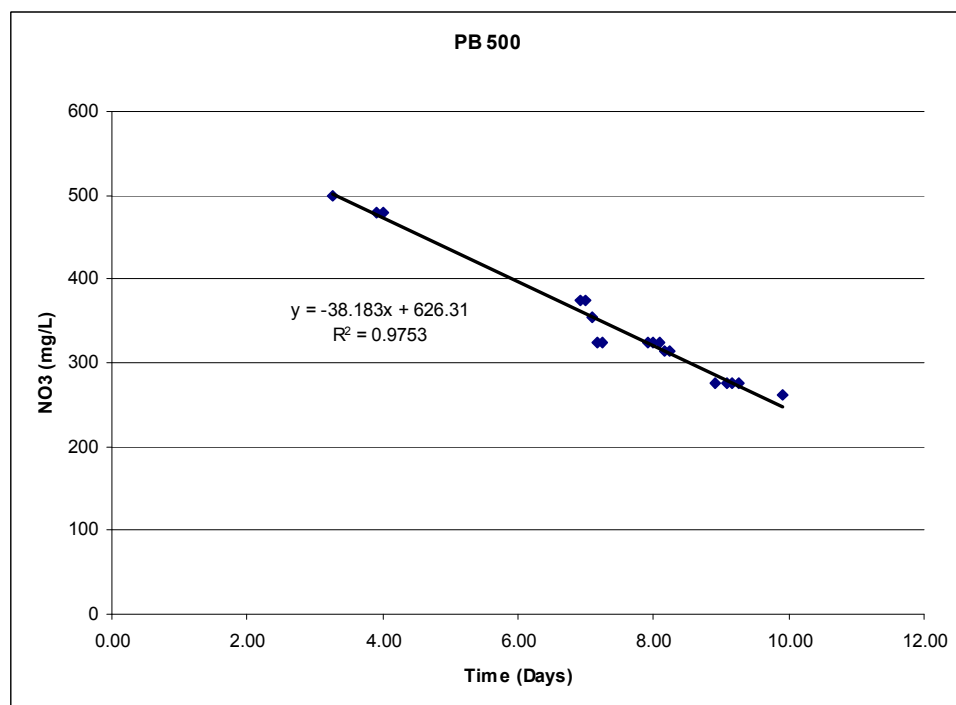


Figure 4.5: Kinetics of PB at $C_o = 500 \text{ mg/}\ell$

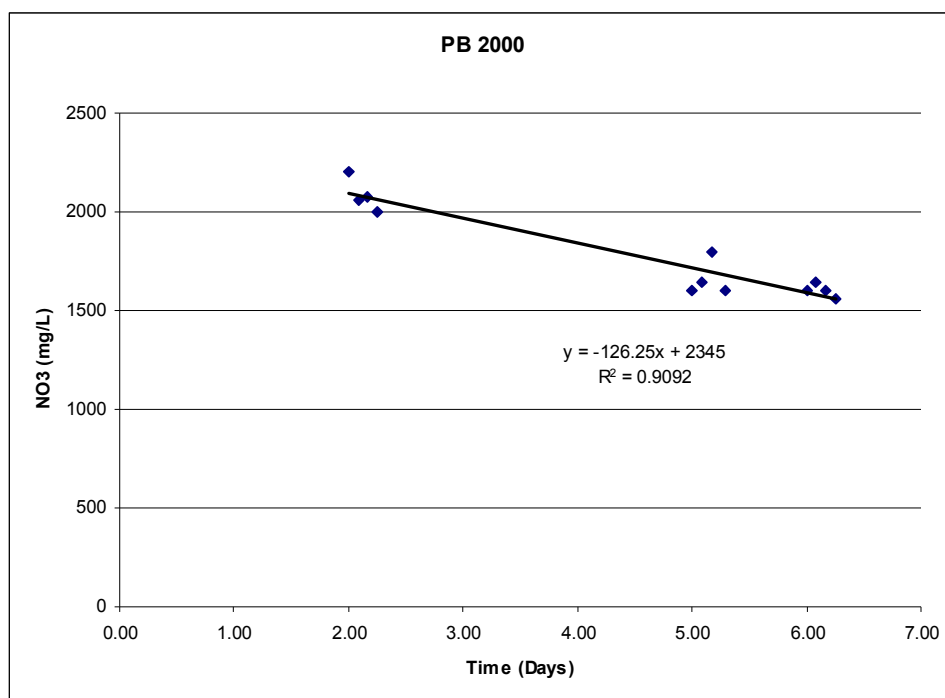
Figure 4.6: Kinetics of PB at $C_0 = 2000 \text{ mg/l}$

Table 4.4 summarises the kinetic rates of removal over the linear period of each batch test, determined from the plotted figures as well as time required to achieve the indicated percent of removal of the PB substrate at the various nitrate concentrations.

Table 4.4: Summary of kinetics of the PB batch tests

$C_0 \text{ (mg/l)}$	Time for 100% Removal (Days)	k (1/day)	R^2	Percentage Removal (%)
100	2.2	46.775	0.98	100
500	-	38.183	0.98	55
2000	-	126.250	0.91	20

All three tests conducted at the various concentration levels showed an initial plateau an acclimatisation period during which there is pH buffering as well as competition between nitrifiers and denitrifiers, as suggested by previous studies (Trois et al., 2009). This period lasted until the environment became more suitable for the denitrifiers. The duration of this plateau period tended to increase with an increase in initial nitrate concentration (Trois et al., 2009).

The test performed at 100 mg/ℓ was the only one to achieve full nitrate removal.

The test conducted at 100 mg/ℓ showed positive results, with total nitrate removal being achieved within 2 – 2.5 days. The tests conducted at 500 and 2000 mg/ℓ showed an increase in nitrates within the first 2 days. This could be due to the small percentage increase represented in the blank as well as errors associated with the method.

The results of the experiment performed at 500 mg/ℓ and 2000 mg/ℓ were less promising, although some removal did occur after the plateau period, full denitrification was not achieved, but only 55% and 20% removal efficiency was observed for the two concentrations respectively.

During the test at 500 mg/ℓ, after 12 to 14 days no more nitrate removal was achieved. This may be due to the inhibitory effect of NO_3 saturation as a result of the high initial nitrate concentration as well as the release of phenols which are toxic to bacteria (De Combret, 2009). Through studies done by De Combret (2009), it is reported that denitrifiers are only present after 74 hours from commencement of the batch test. Thus the removal of nitrate within 2.2 days at a concentration of 100 mg/ℓ could be attributed to absorption of nitrates or the reduction of nitrates into ammonia (Trois et al., 2010).

The test conducted at 2000 mg/ℓ showed little nitrate removal. After the plateau period, the nitrate concentration did decrease by 20 – 30%, but after the initial 5 days further reduction was no longer achieved and the final concentration stabilised at 1600 mg/ℓ.

4.3.2. Fresh and Composted Garden Refuse

4.3.2.1. Fresh commercial garden refuse (CGR RAW).

Table 4.5 presents the results of the characterisation of inputs and outputs materials from the batch tests with CGR RAW.

Table 4.5: Characterisation results of the input and output of the CGR RAW batch tests

Parameter	Input Eluate	Input Solid	Blank (0 mg/l)		100 (mg/l)		500 (mg/l)		2000 (mg/l)	
			Output Eluate	Output Solid	Output Eluate	Output Solid	Output Eluate	Output Solid	Output Eluate	Output Solid
pH	5.45		6.01		5.97 – 6.16		5.41 – 5.68		6.80 – 7.33	
COD (mg/l)	4253		9433		4325 – 5212		3951 – 7200		7009 – 7870	
NH ₃ -N (mg/l)	12.74		15		4 – 30		20 – 30		75 – 100	
NO ₃ (mg/l)	6.86		0		0					
Total C (%)	0.083	49.6		48.5		42.9 – 47.6		46.4 – 48.8		45.6 – 49.5
Total N (%)	0.0183	0.55		0.63		0.57 – 0.84		0.70 – 0.84		0.19 – 0.68
C/N Ratio	4.54	90.19		76.98		54.64 – 75.26		55.79 – 70.25		67.5 – 240.0

Due to the large number of tests carried out at each concentration, an average value would have provided a misrepresentation of the results.

It is noted that the fresh CGR can be compared with the pine bark in terms of pH that ranges around 5.45 and increases with time and with NO₃ concentration as reported by other authors (Tsui et al., 2007). It is also noted that the longer test conducted at an initial concentration of 2000 mg/l exhibits a final pH which falls into the optimum range for denitrification.

To monitor the NO₂ concentrations during the 500 mg/l experiment, three tests were stopped at different levels of nitrites. The 500 – A test had a much lower amount of NO_x-N whereas the test that was stopped when nitrites were still present had a relatively high value of NO_x-N.

Table 4.6: Characterisation results of the output of the CGR RAW batch tests conducted at 500 mg/l on both solid and eluate

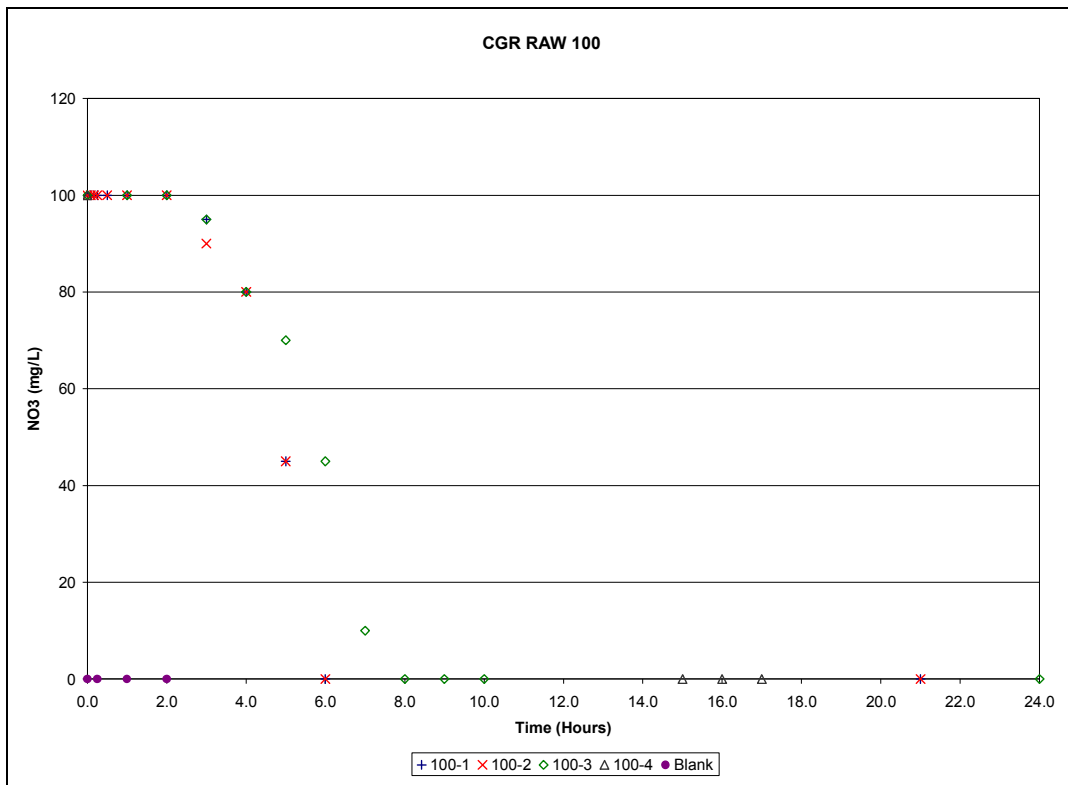
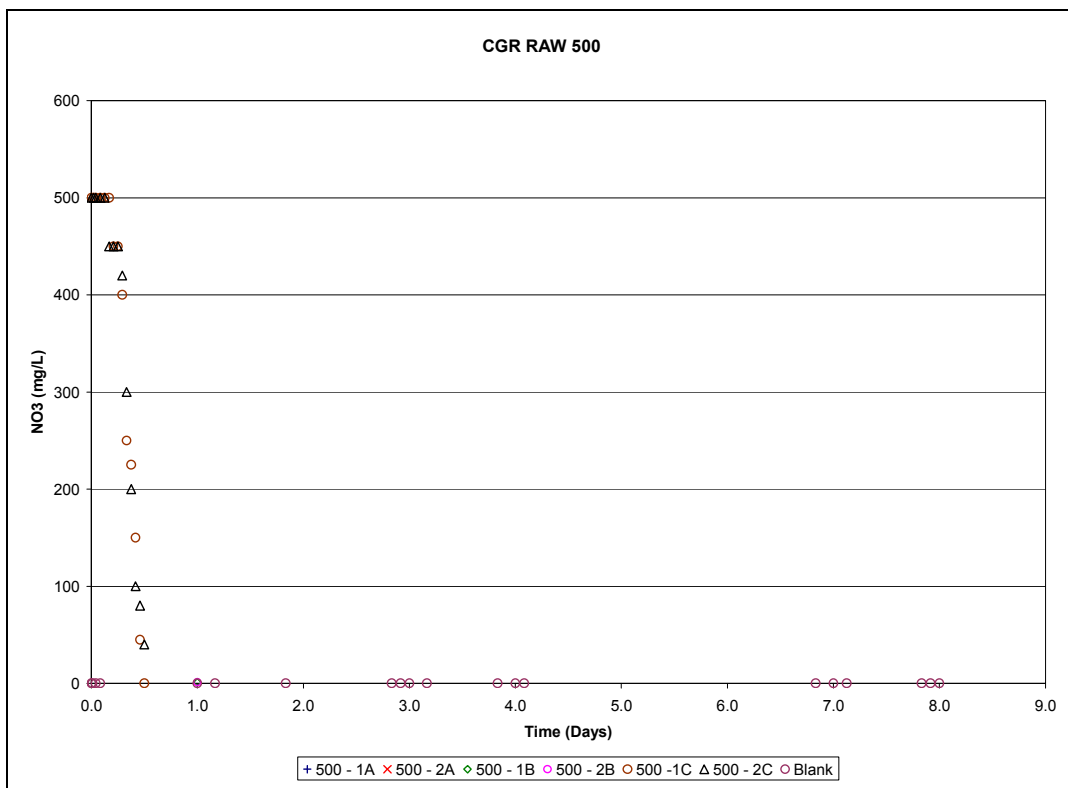
	CGR RAW (500 - A)	CGR RAW (500 - B)	CGR RAW (500 - C)
Solid			
Total C (%)	48.4	46.4	48.8
Total N (%)	0.72	0.84	0.7
C/N Ratio	67.89	55.79	70.25
Eluate			
pH	5.41	5.68	5.47
COD	7200	3951	4046
NH₃-N	30.0	25.0	20.0
NO_x-N	3.0	85.0	62.5

As a result of the production of NH₃ leached out from the substrate as well as the oxygen present in the solution and the pores, NH₃ is converted into NO₂ even when full nitrate removal is achieved. It was confirmed by De Combret (2009) and Trois (2010) that both nitrifiers and denitrifiers were present in this substrate within the first 74 hours of batch test, in line with other studies that used similar substrates (Zhong et al., 2009)

There was a presence of positive bioleaching of carbon which was observed in the increase of both the COD and C/N ratios, relating to the initial nitrate concentration. The COD results showed an increase from the initial input ranging from 3951 – 7870 mg/l. The ammoniacal nitrogen released, also tended to increase with the time. This increase in NH₃ which correlates to the slight reduction in total N (%) especially in the test at C₀ = 2000 mg/l, indicates that there was also bioleaching of nitrogen. As the percentage increase in COD was not as great as that observed in the PB, there was a lower increase in C/N ratio. As C/N ratio was calculated using wet samples, carbon leached out from the substrate was still trapped in the biofilm of the pores resulting in the observed increase in C/N Ratio from 90.19 to 240.0.

The evolution of the nitrate concentration for the tests with CGR RAW substrate conducted for each of the concentrations is shown in Figures 4.7, 4.8, 4.9 and 4.10.

The blank test results are also included for reference.

Figure 4.7: Evolution of the nitrate concentrations for CGR RAW at $C_0 = 100 \text{ mg/}\ell$ Figure 4.8: Evolution of the nitrate concentration for CGR RAW at $C_0 = 500 \text{ mg/}\ell$

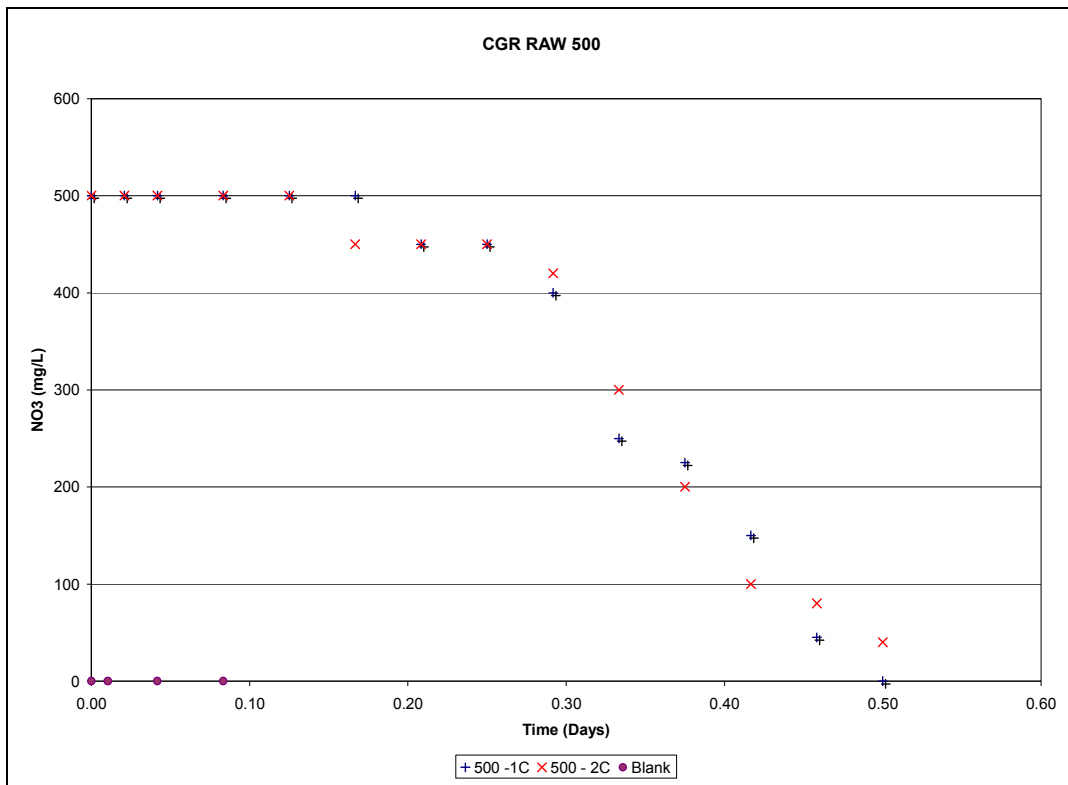
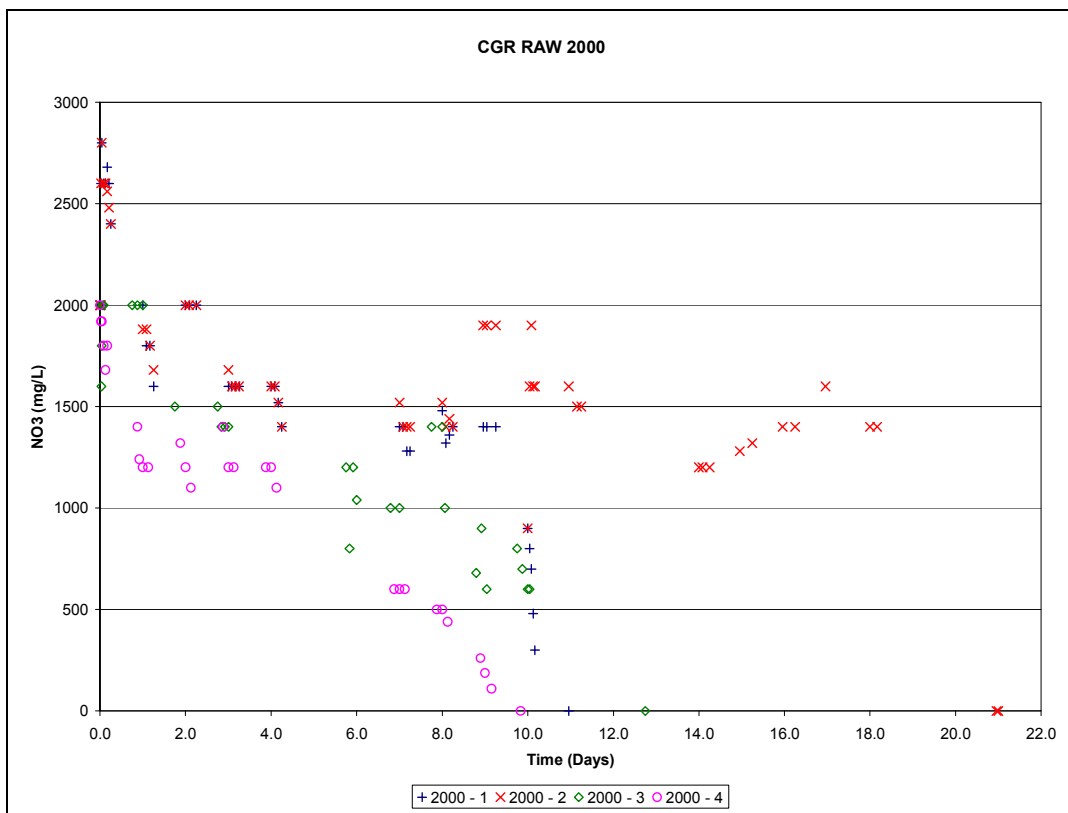


Figure 4.9: Evolution of the nitrate concentration for CGR RAW at 500 mg/l (Test C)

Figure 4.10: Evolution of the nitrate concentration for CGR RAW at $C_0 = 2000$ mg/l

Kinetics: Rate of Reaction

The results were modelled using a zero order kinetic reaction model.

Rate of Reaction for linear period:

100 mg/l: Highest (Zero Nitrates - 1)

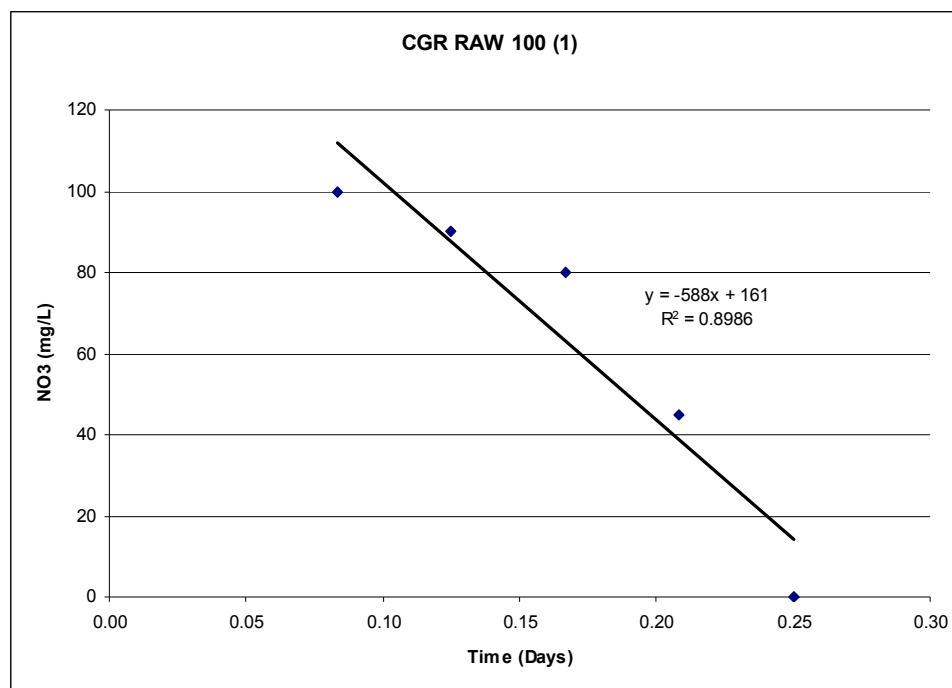


Figure 4.11: Kinetics of CGR RAW at $C_o = 100 \text{ mg/l}$ (1)

100 mg/l: Lowest (Zero Nitrates and Nitrites - 2)

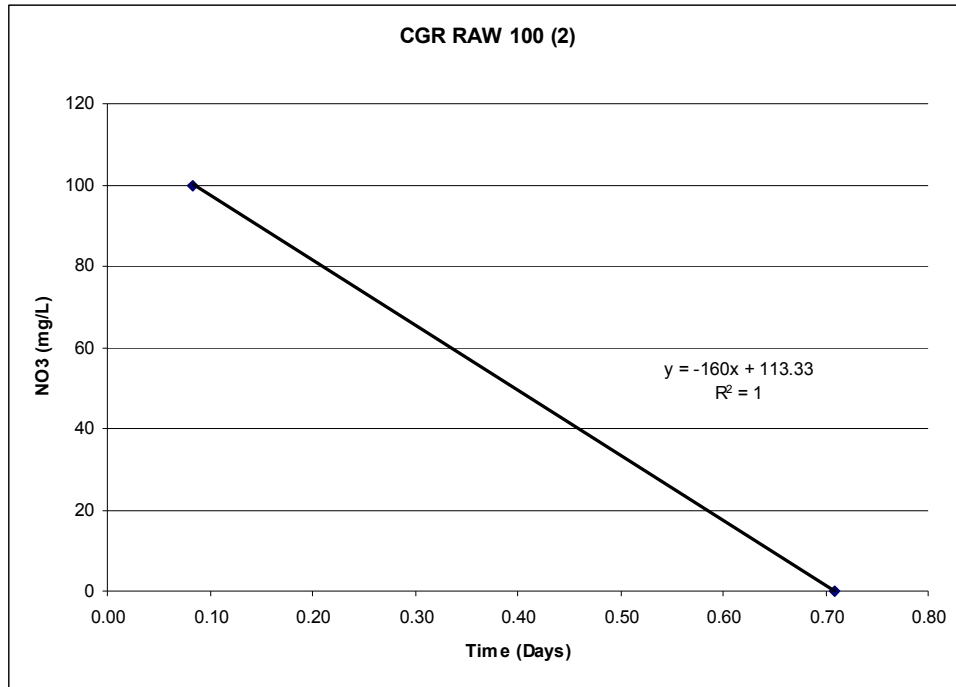


Figure 4.12: Kinetics of CGR RAW at $C_0 = 100 \text{ mg/l}$ (2)

500 mg/l: Highest (Zero Nitrates - 1)

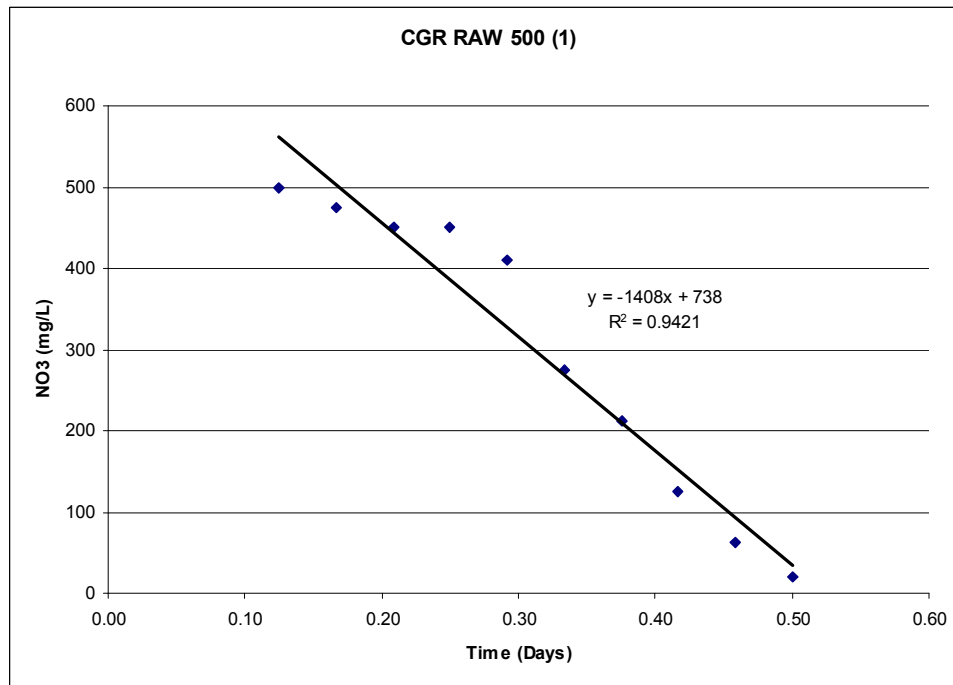


Figure 4.13: Kinetics of CGR RAW at $C_0 = 500 \text{ mg/l}$ (1)

500 mg/l: Lowest (Zero Nitrates and Nitrites - 2)

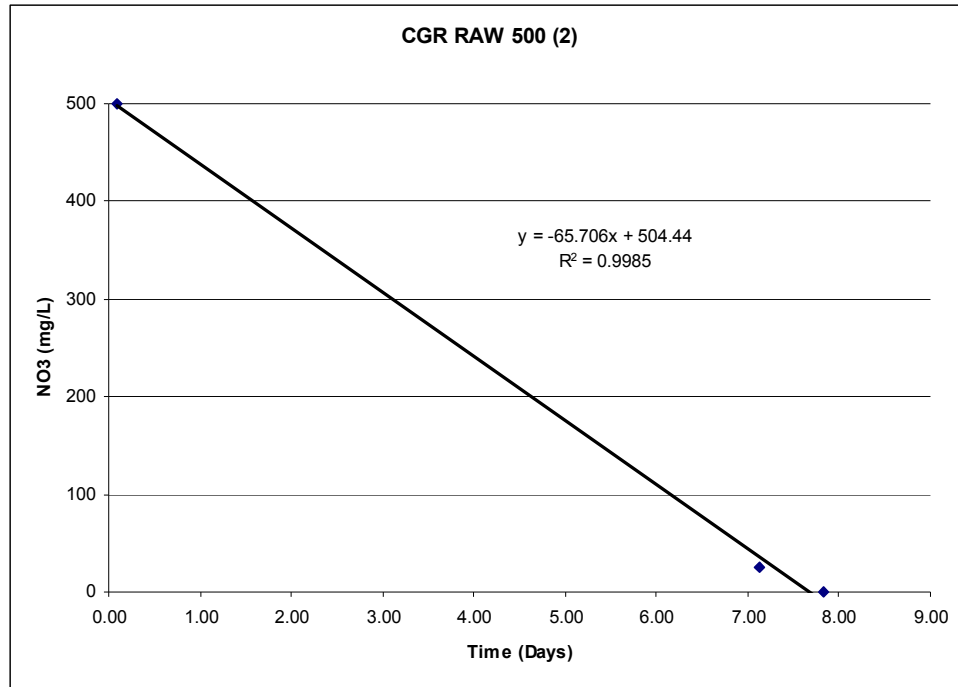


Figure 4.14: Kinetics of CGR RAW at $C_0 = 500 \text{ mg/l}$ (2)

2000 mg/l:

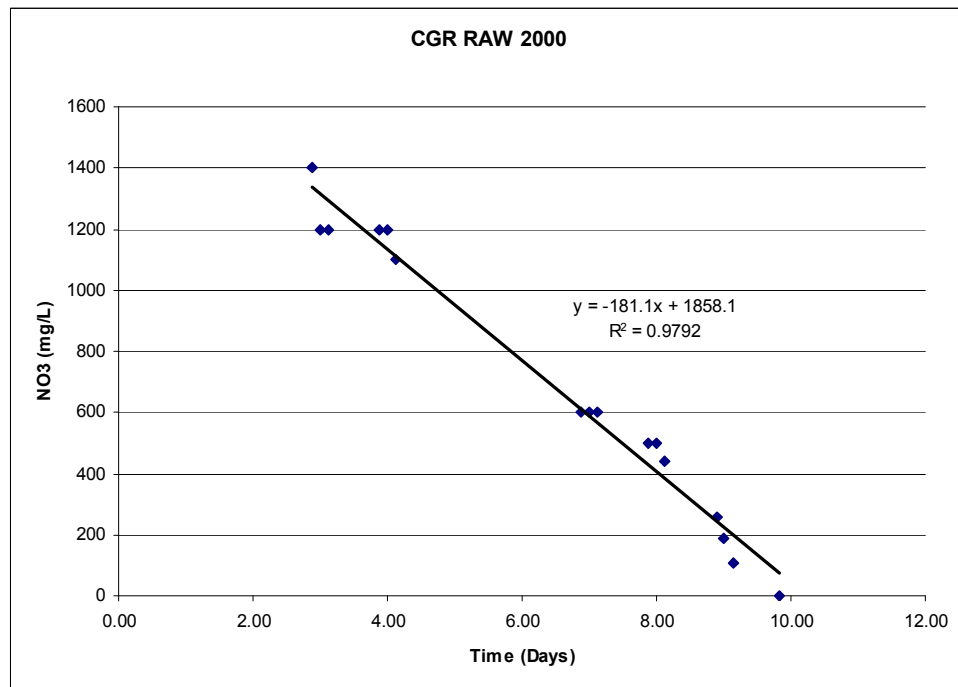


Figure 4.15: Kinetics of CGR RAW at $C_0 = 2000 \text{ mg/l}$

Table 4.7 summarises the kinetic rates of removal over the linear period of each batch test, determined from the plotted figures as well as time required to achieve the indicated percent of removal of the CGR RAW substrate at the various nitrate concentrations. 100 (1) is the time for the removal of all nitrates whereas 100 (2) is the period for the removal of both the nitrites and nitrates, similarly for 500 (1) and 500 (2).

Table 4.7: Summary of kinetics of CGR RAW

C_o (mg/l)	Time for 100% Removal (Days)	K (1/day)	R²	Percentage Removal (%)
Removal of nitrates only				
100 (1)	0.25	588	0.90	100
500 (1)	0.50	1408	0.94	100
2000	10.5	181	0.98	100
Removal of nitrates and nitrites				
100 (2)	0.71	160	1.00	100
500 (2)	7.83	67.71	0.999	100

All three tests conducted at the various concentration levels exhibited an initial plateau of approximately 2 hours. Similarly to the Pine Bark substrate, which also experiences an acclimatisation period, this involves pH buffering. The duration of this plateau period tended to increase with an increase in initial nitrate concentration, suggesting that pH and the initial NO₃ concentration play an important inhibitory role during this initial stage as demonstrated by De Combret (2009).

In the test at C_o = 100 mg/l the system reached a zero nitrate concentration within 6 to 8 hours with a 2 hour plateau. A total of 4 tests were performed at this concentration to accurately obtain the time required for complete nitrate removal.

The tests conducted at C_o = 500 mg/l demonstrated an initial plateau period ranging between 2 to 8 hours. After this plateau the nitrate concentration rapidly dropped to zero after 12 hours. Once again, as experienced in the test conducted at 100 mg/l there were nitrites present after the nitrate concentration became zero, with zero nitrates and nitrites present after 8 days.

The final test at a concentration of $C_0 = 2000 \text{ mg/l}$ showed an increase in nitrates within the first 6 hours of the initial two tests and a plateau period of 18 to 24 hours with full nitrate removal occurring from 9 to 12 days.

One of the tests behaved slightly differently ($2000 - 2$). It showed an initial peak followed by a similar plateau stage. The nitrate concentration then decreases at a rapid rate until a concentration of 1400 mg/l after 4 days was reached. The fluctuations in the nitrate concentrations are not fully understood. Finally at approximately 18.5 days, the nitrate level dropped from 1400 mg/l in two days to zero.

All the tests reach 100% removal. The tests conducted at 100 and 500 mg/l were both highly efficient and reached a zero nitrate concentration in less than 24 hours. The graphical representations suggest a linear relationship, excluding the initial plateau period. Studies done by De Combret (2009) and Trois (2010) suggest that denitrifiers are only present after 74 hours, thus the removal of nitrate within 24 hours could be attributed to other bio-chemical processes such as absorption of nitrates or the conversion of nitrates into ammonia.

From the above results it is possible to conclude that this substrate is suitable for biological denitrification.

2. Immature Compost: Domestic Garden Refuse 10 Weeks (DGR 10)

Table 4.8 shows the characterisation results of the tests performed on the inputs and outputs in the batch tests (DGR 10).

Table 4.8: Characterisation results of the input and output of the DGR 10 batch tests.

Parameter	Input Eluate	Input Solid	Blank (0 mg/l)		100 (mg/l)		500 (mg/l)	
			Output Eluate	Output Solid	Output Eluate	Output Solid	Output Eluate	Output Solid
pH	7.40		7.41		7.33		7.55	
COD (mg/l)	17556		19820		7822		17783	
NH ₃ -N (mg/l)	82.04		30		8.5		87.2	
NO ₃ (mg/l)	15.20		0		0		0	
Total C (%)	1.0	23.97		24.3		25.8		49.6
Total N (%)	0.12	1.88		1.71		1.6		0.55
C/N Ratio	8.3	12.75		14.21		16.13		90.19

The pH remained constant around the optimum range for denitrification for all tests.

All the tests achieved a zero nitrate (NO₃) concentration at the end of the test.

The COD results are similar to the input value; however the test conducted at 100 mg/l showed a substantial drop, which is promising.

It is noted that the initial input material had a high NH₃ - N value. The shorter test conducted at 100 mg/l showed a drastic decrease of 90%. The longer tests, the blank and 500 mg/l, still showed a high value at the end of the tests, with the 500 mg/l increasing above that of the initial input. This increase in NH₃ correlates to the reduction in total N (%) from 1.88 – 0.55, which indicates there is also bioleaching of nitrogen.

There was a presence of positive bioleaching of carbon which was observed in the increase of the C/N ratios, relating to the initial nitrate concentration, where the C/N ratio of the C₀ = 500 mg/l test increased by approx. 600% of that of the initial input. As C/N ratio was calculated using wet samples, carbon leached out from the substrate was still trapped in the biofilm of the pores resulting in the observed increase in C/N Ratio from 12.75 to 90.19.

The evolution of the nitrate concentration for the DGR 10 substrate conducted for each of the initial concentrations is shown in Figures 4.16, 4.17, 4.18 and 4.19.

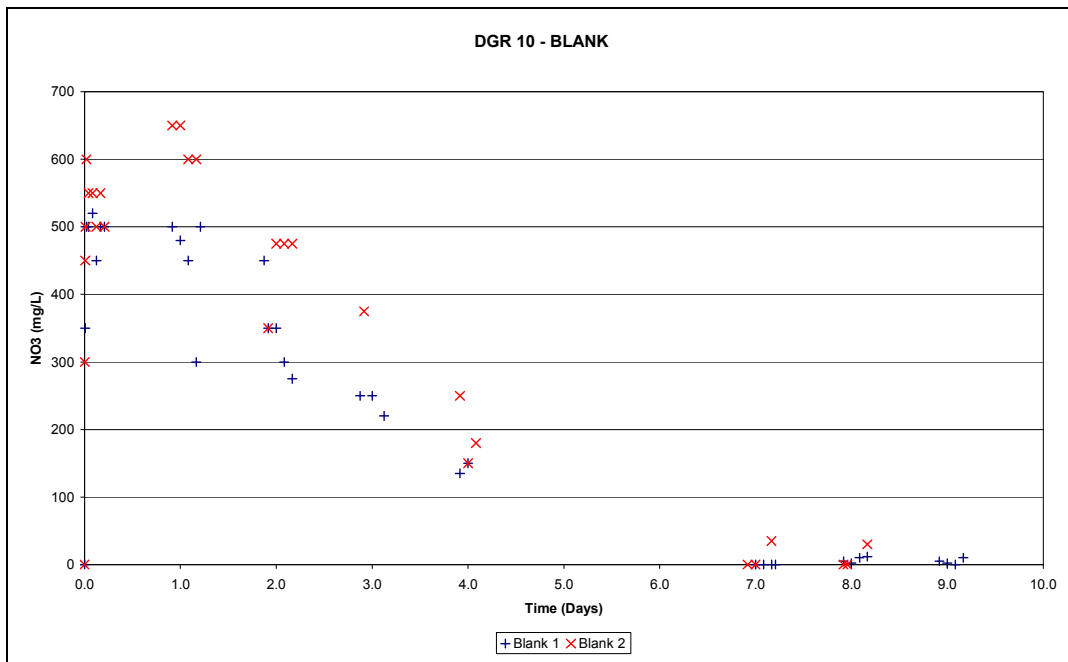


Figure 4.16: Evolution of the nitrate concentration for DGR 10 Blank at $C_0 = 0 \text{ mg/l}$

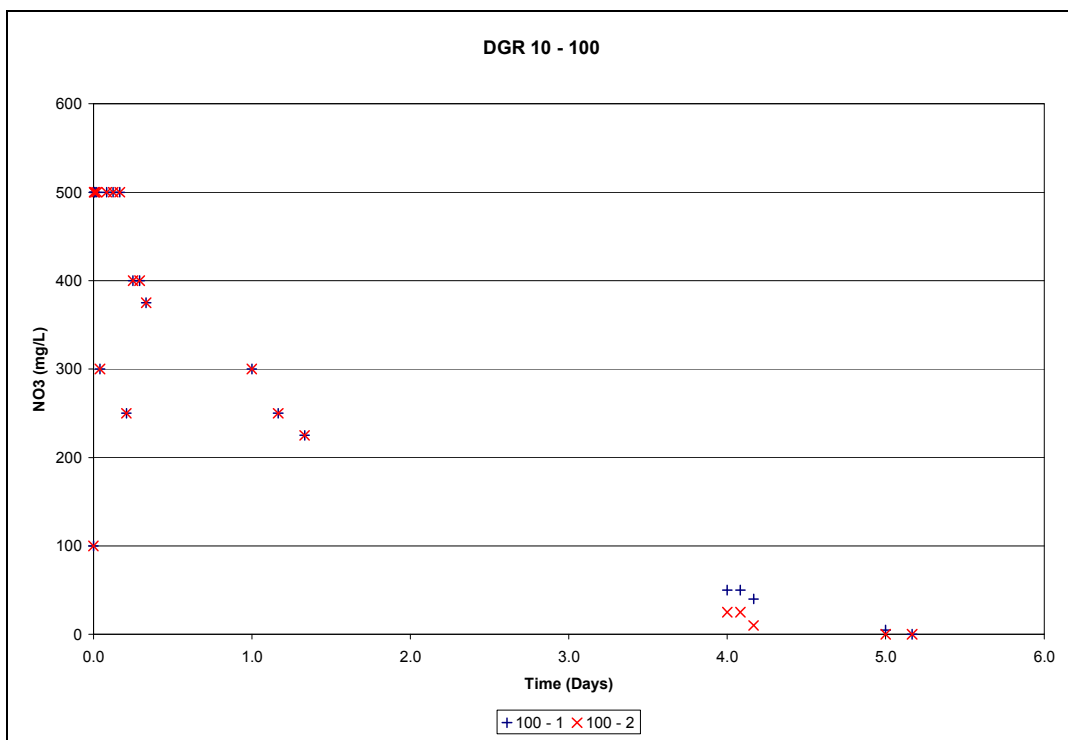
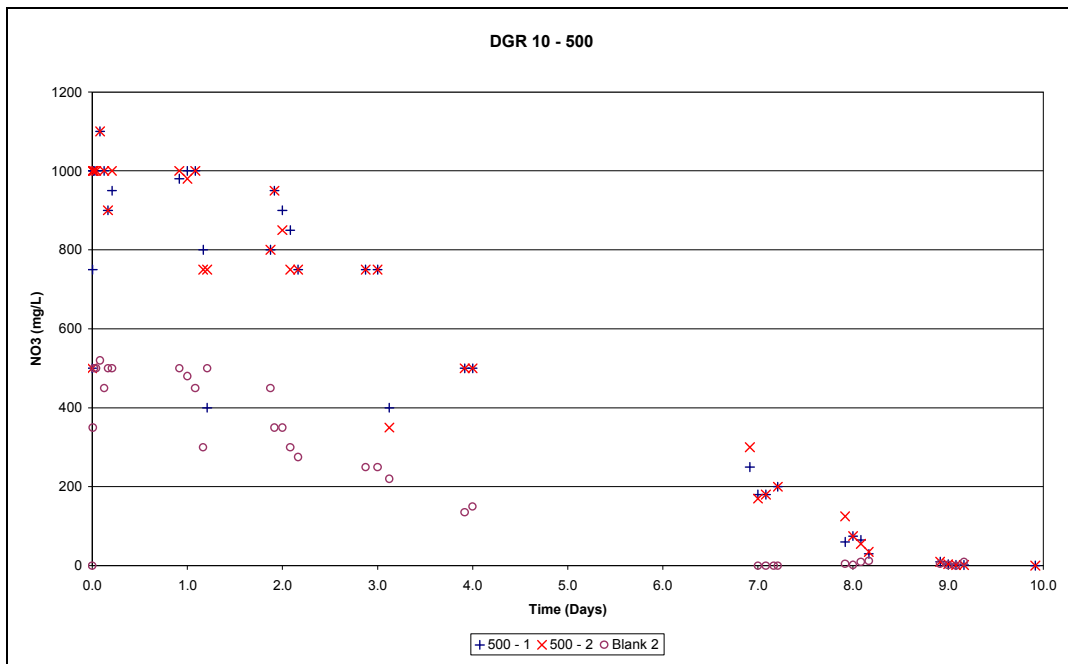
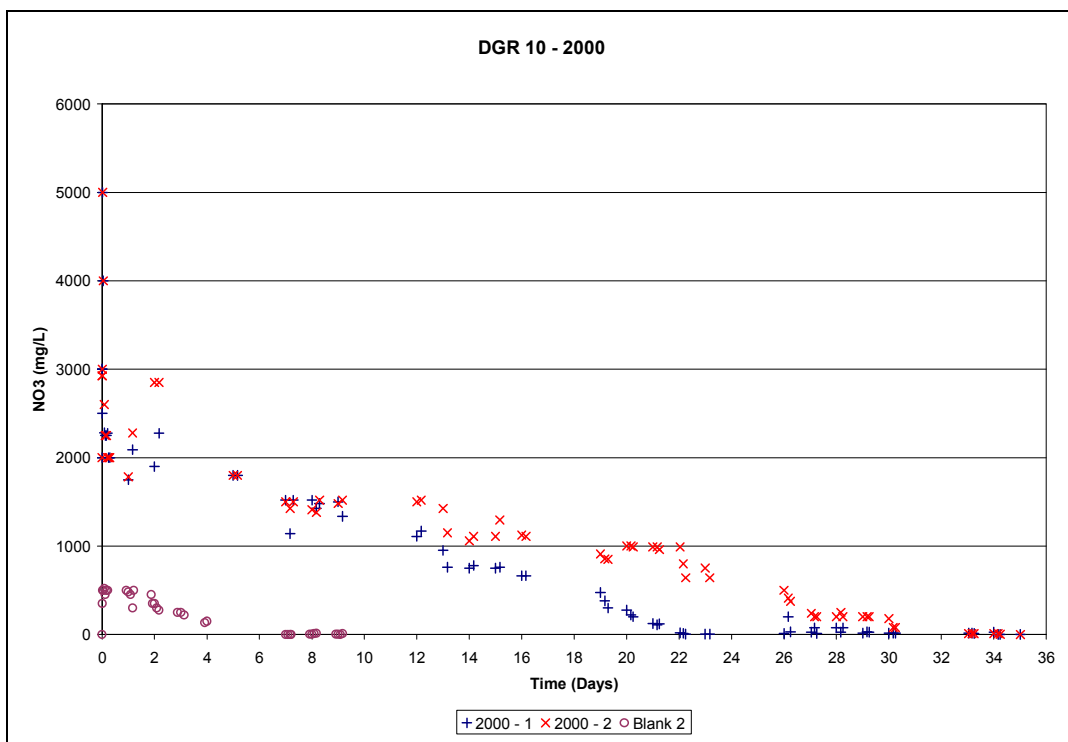


Figure 4.17: Evolution of the nitrate concentration for DGR 10 at $C_0 = 100 \text{ mg/l}$

Figure 4.18: Evolution of the nitrate concentration for DGR 10 at $C_0 = 500 \text{ mg/l}$ Figure 4.19: Evolution of the nitrate concentration for DGR 10 at $C_0 = 2000 \text{ mg/l}$

Kinetics: Rate of Reaction

The following figures summarise the modelling of the kinetic rates of removal over the linear period of each batch test with the best fit line being applied. The results were modelled using both a linear and exponential relationship and it was found that a zero order reaction provided a more accurate representation.

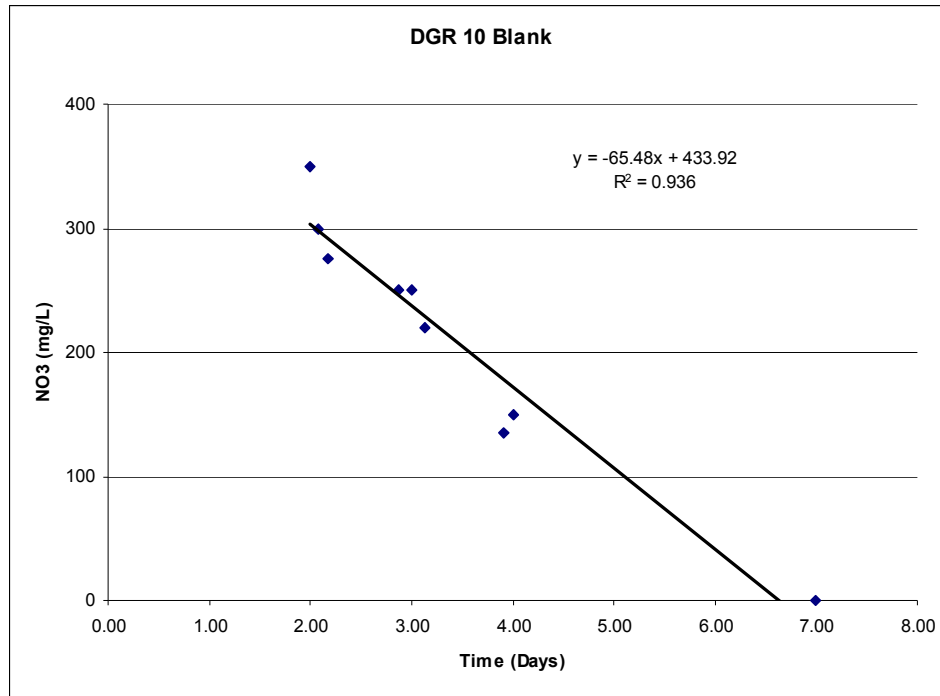
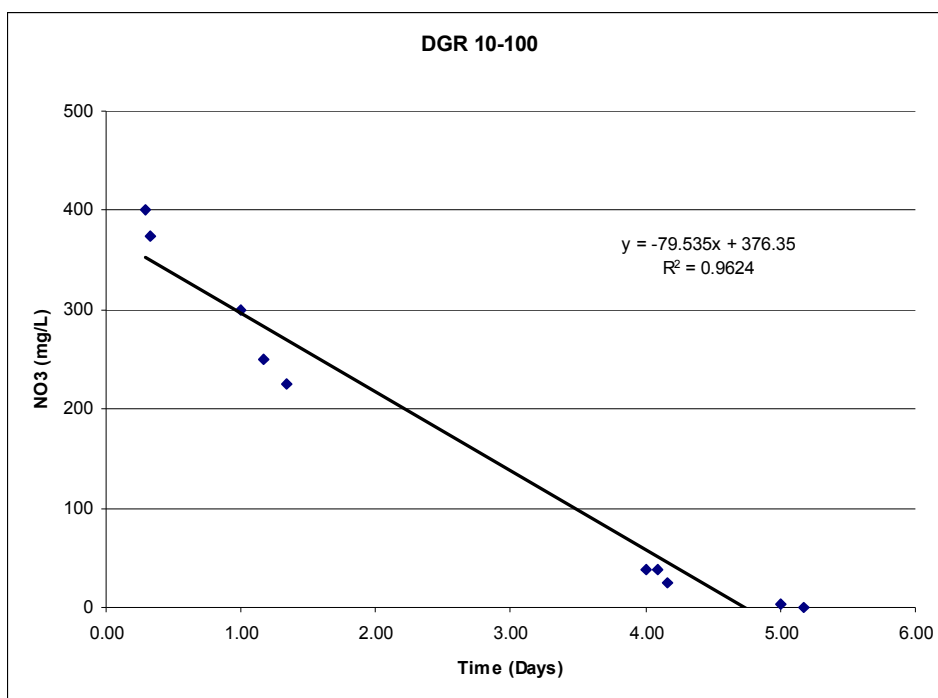
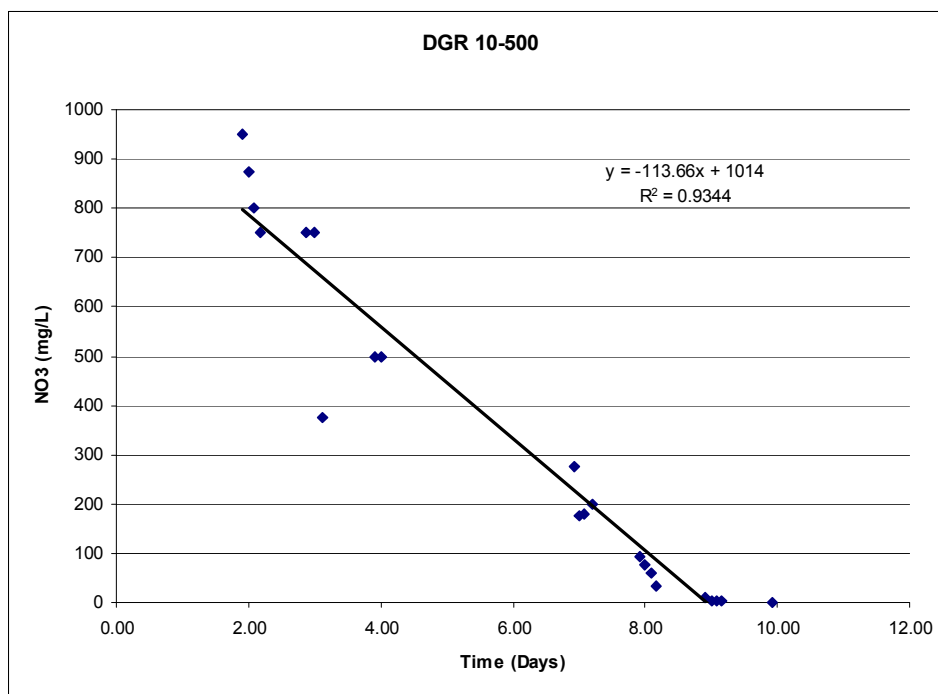


Figure 4.20: Kinetics of DGR 10 at $C_o = 0 \text{ mg/l}$

Figure 4.21: Kinetics of DGR 10 at $C_0 = 100 \text{ mg/l}$ Figure 4.22: Kinetics of DGR 10 at $C_0 = 500 \text{ mg/l}$

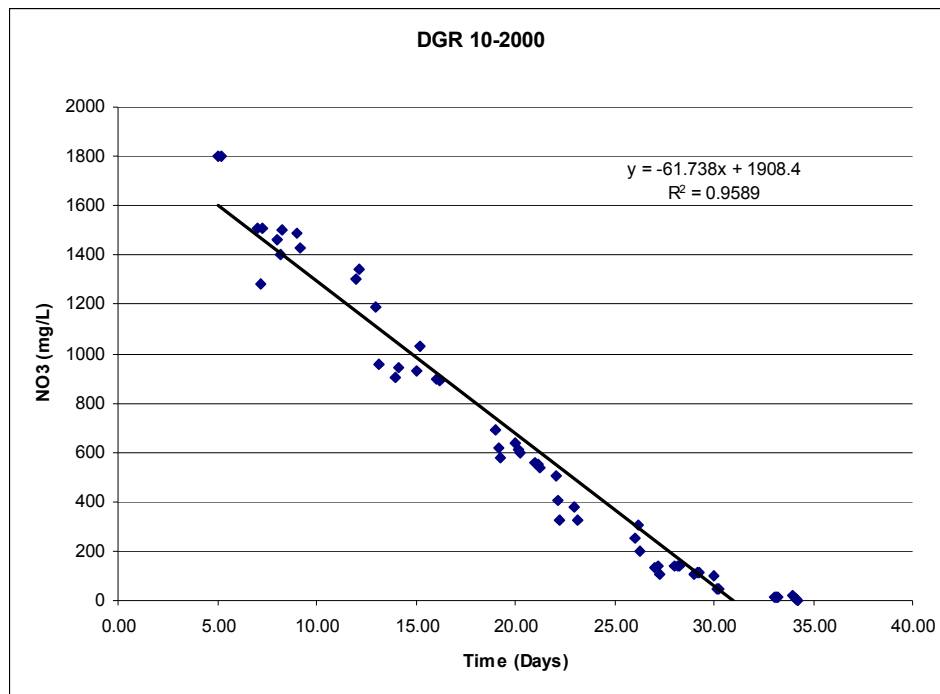
Figure 4.23: Kinetics of DGR 10 at $C_0 = 2000 \text{ mg/}\ell$

Table 4.9 summarises the kinetic rates of removal over the linear trend of each batch test.

Table 4.9: Summary of kinetics of DGR 10

C_0 (mg/ℓ)	Time for 100% Removal (Days)	k (1/day)	R^2	Percentage Removal (%)
Blank	9	65.48	0.94	100
100	5	79.74	0.96	100
500	9.5	113.66	0.93	100
2000	34.5	61.74	0.96	100

The blank test provided some very interesting results. The nitrate concentration actually increased significantly within the first two days of the test ranging between 500 and 650 mg/ℓ. A small plateau was experienced at this high concentration for approximately 1.5 days. The denitrification process then followed a linear relationship until full nitrate removal was achieved after 8 to 9 days. This initial increase in nitrates was first believed to be due to added nutrients used by domestic households, such as fertilizers. However after examining the input and output results, it is concluded that the considerable rise in nitrates was more likely due to organic nitrates and ammoniacal nitrogen from bioleaching of the organic nitrogen from the solid substrate matter rather

than nitrification. From Table 4.8 the initial input material has relatively high values of both $\text{NH}_3\text{-N}$ and $\text{NO}_x\text{-N}$. The increase in nitrate concentration also correlates to the reduction in total N (%). As carbon and nitrogen are leached from the matter, denitrification is limited by the availability of electron donors and thus there was an increase in nitrate concentration (Tsui et al.; 2007).

All the tests showed a similar trend as that of the blank test. An initial rise in nitrates occurs due to the relatively high values of both $\text{NH}_3\text{-N}$ and $\text{NO}_x\text{-N}$ in the input material. After this rise a plateau period is established as the test reached its regime, followed by a rapid rate of denitrification which reduces the nitrate concentration to zero.

In the case of the 100 mg/l test a plateau of 4 hours is observed with full nitrate removal after 5 days. In the 500 mg/l test, a plateau was once again experienced for 2 to 3 days before total nitrate removal after 9 – 10 days. The final test performed at 2000 mg/l again displayed a plateau period of 2 – 3 days and reached zero nitrate concentration after 34.5 days at a linear rate.

3. Immature Compost: Commercial Garden Refuse 10 Weeks (CGR 10)

Table 4.10 shows the characterisation results of the tests performed on the inputs and outputs in the batch tests (CGR10).

Table 4.10: Characterisation results of the input and output of the CGR 10 batch tests.

Parameter	Input Eluate	Input Solid	Blank (0 mg/l)		100 (mg/l)		500 (mg/l)	
			Output Eluate	Output Solid	Output Eluate	Output Solid	Output Eluate	Output Solid
pH	6.98		7.08		7.22		7.51	
COD (mg/l)	2764		1944		2754		3177	
$\text{NH}_3\text{-N}$ (mg/l)	9.80		7.0		2.5		3.0	
NO_3 (mg/l)	7.14		0		0		0	
Total C (%)	0.11	28.69		45.2		45.2		41.9
Total N (%)	0.06	1.20		0.94		0.49		1.23
C/N Ratio	1.83	23.91		48.9		92.24		34.07

The pH values throughout the tests increased with the increase of the initial concentration and remain constant to optimum ranges for denitrification (Trois et al., 2007). There was a presence of positive bioleaching of carbon which was observed in the increase of the COD, relating to the initial nitrate concentration. The COD results showed an increase from the initial input ranging from 2764 – 3177 mg/l. The $\text{NH}_3\text{-N}$

values in all the tests were lower than that of the initial input material. The test conducted at 100 and 500 mg/l showed a drastic decrease of 70 - 75%.

The percentage carbon for all tests increased for all experiments falling in the range of 41 – 46%. The percentage of nitrogen remained constant throughout all the experiments apart for the 100mg/l test where the output %N was more than 50% less than the input material resulting in the C/N ratio being noticeably higher than the rest. The C/N ratios for the tests were all higher than the input value. This is a result of the C/N ratio being calculated using wet samples, thus carbon leached out from the substrate was still trapped in the biofilm of the pores.

The evolution of the nitrate concentration for the CGR 10 substrate conducted for each of the initial concentrations is shown in Figures 4.24, 4.25 and 4.26.

The blank test showed no leaching out of nitrates; however its results are still included in each of the graphs.

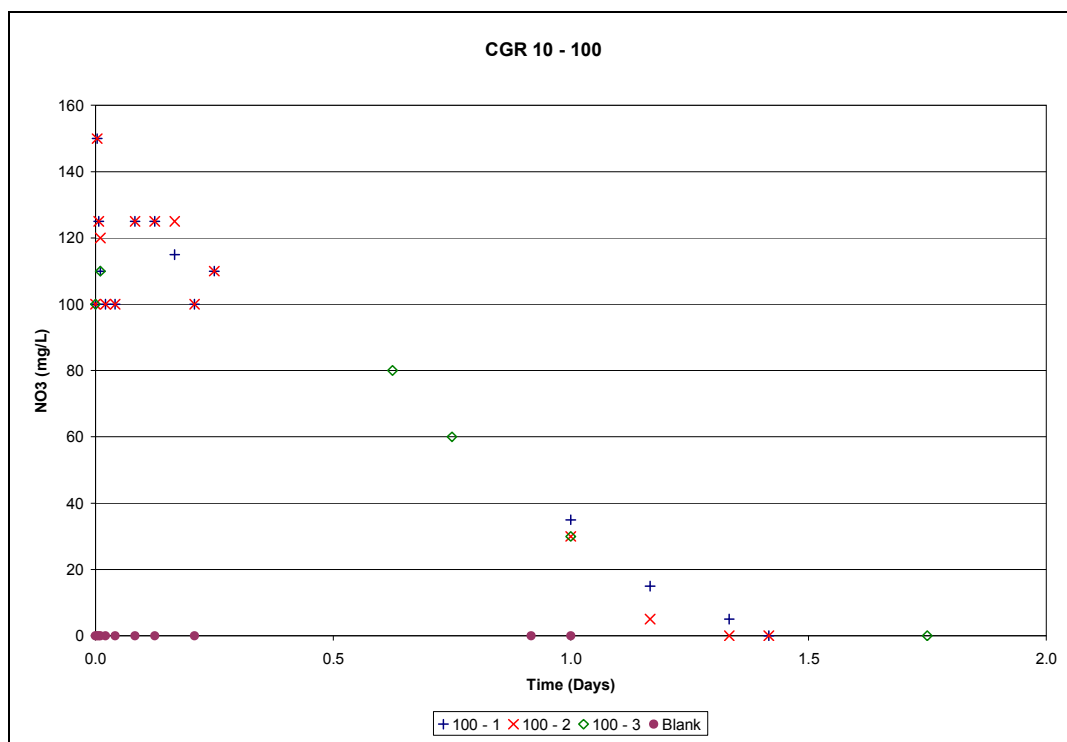
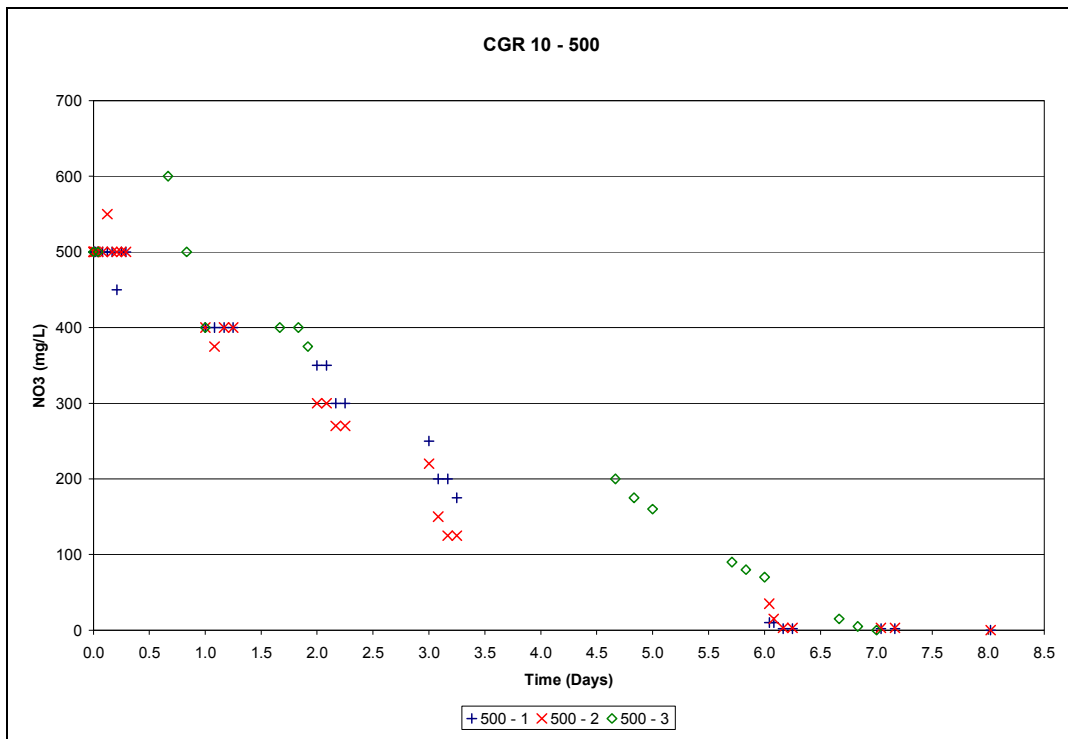
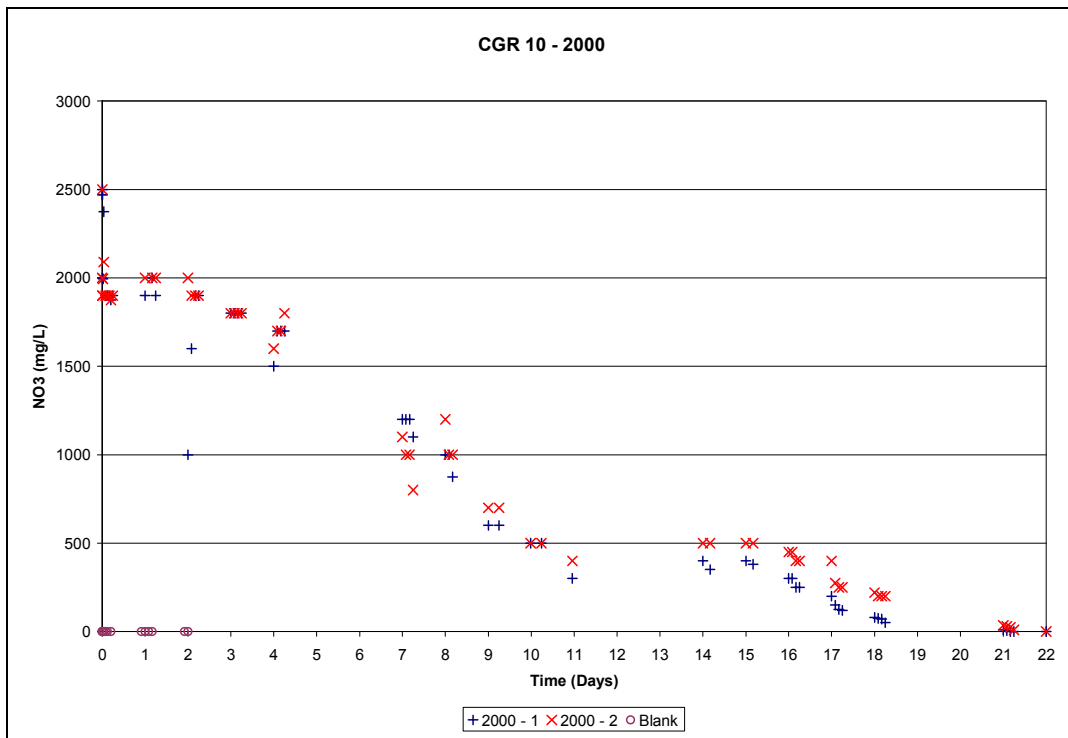


Figure 4.24: Evolution of the nitrate concentration for CGR 10 at $C_0 = 100$ mg/l

Figure 4.25: Evolution of the nitrate concentration for CGR 10 at $C_0 = 500 \text{ mg/}\ell$ Figure 4.26: Evolution of the nitrate concentration for CGR 10 at $C_0 = 2000 \text{ mg/}\ell$

Kinetics: Rate of Reaction

The results were modelled using a zero and first order kinetic reaction model.

Zero order reaction: $\frac{dc}{dt} = -k \rightarrow c = c_o - kt$

First order reaction: $\frac{dc}{dt} = -kC \rightarrow c = c_o e^{-kt}$

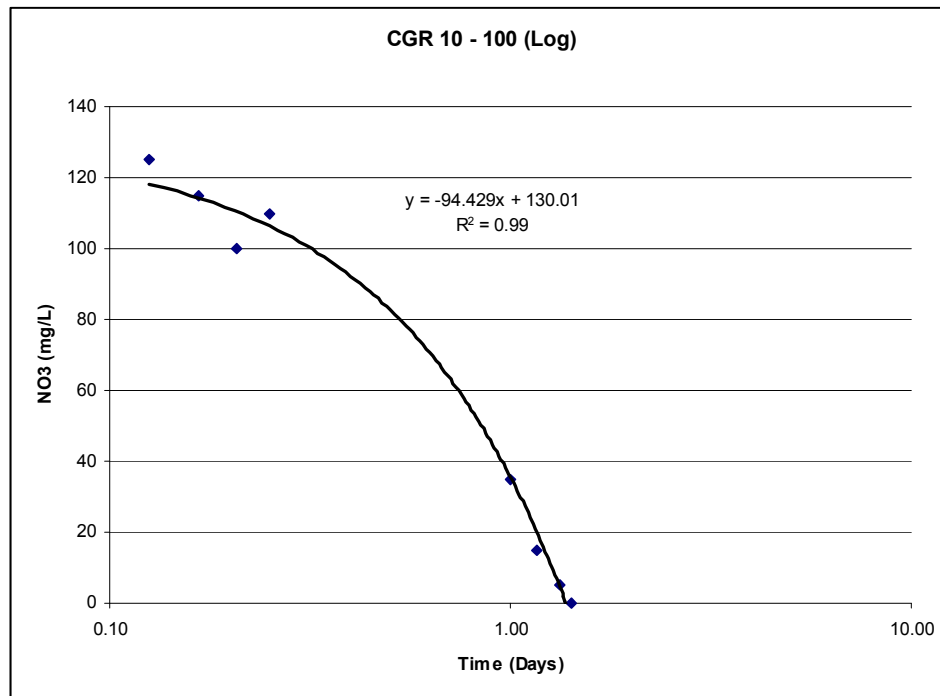
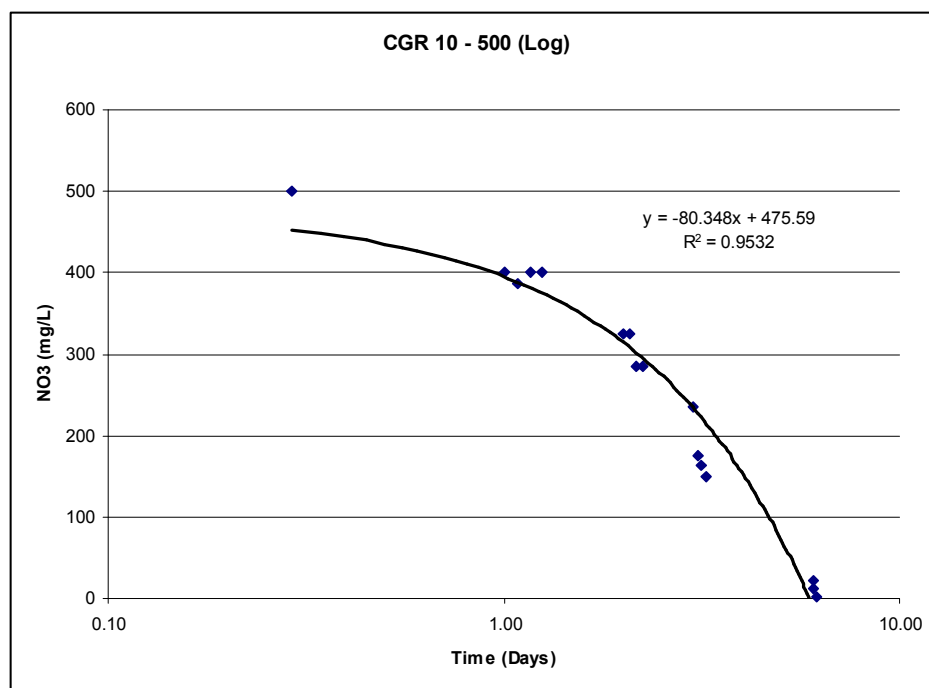
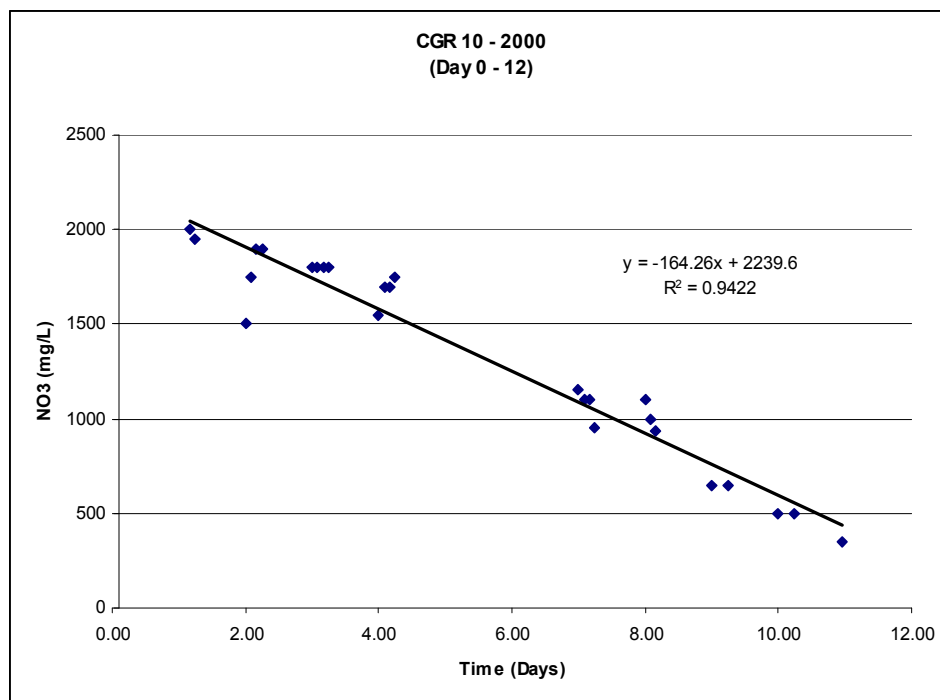


Figure 4.27: Kinetics of CGR 10 at $C_o = 100 \text{ mg/l}$ in Log Scale

Figure 4.28: Kinetics of CGR 10 at $C_0 = 500 \text{ mg/l}$ in Log ScaleFigure 4.29: Kinetics of CGR 10 at $C_0 = 2000 \text{ mg/l}$ [A] (Day 0 - 12)

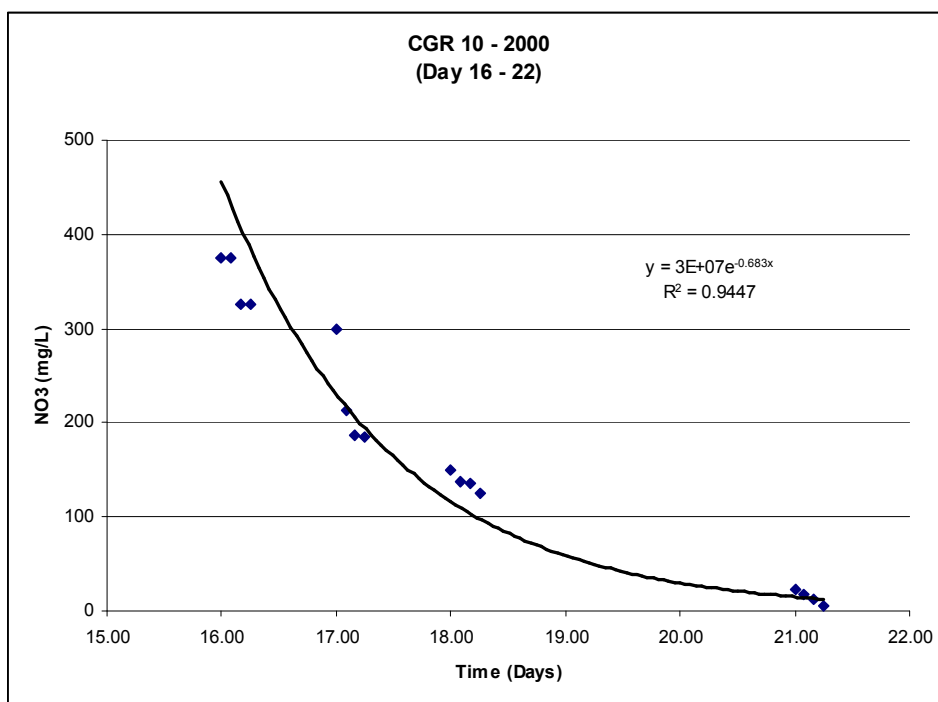
Figure 4.30: Kinetics of CGR 10 at $C_0 = 2000 \text{ mg/l [B]}$ (Day 16 -22)

Table 4.11: Summary of kinetics of CGR 10

C_0 (mg/l)	Time for 100% Removal (Days)	k (1/day)	R^2	Percentage Removal (%)
100	1.5	94.43	0.99	100
500	8	80.35	0.95	100
2000 [A]	22	164.26	0.94	100
2000 [B]	22	0.683	0.94	100

Note: $C_0 = 2000 \text{ mg/l [A]}$ (Day 0 -12) – Linear relationship

$C_0 = 2000 \text{ mg/l [B]}$ (Day 16 -22) – Exponential relationship

Each test presents an acclimatisation period which is dependent on the initial concentration, with the 2000 mg/l test having the longest plateau of 3 - 4 days, followed by 12 days of removal at a linear rate and a final exponential tail after day 16. After the plateau, nitrate removal occurred at a linear rate until a zero nitrate concentration was achieved, between 1.25 to 1.75 days for the 100 mg/l test, 7 to 8 days for the 500 mg/l test and 22 days for the experiment at 2000 mg/l. Microbial tests conducted by De Combret in 2009 suggest that high performance of the test at 100 mg/l could be to other phenomena rather than bio-denitrification.

4. Mature Compost: Dome Aeration Technology (DAT)

Table 4.12 shows the characterisation results of the tests performed on the inputs and outputs in the batch tests (DAT).

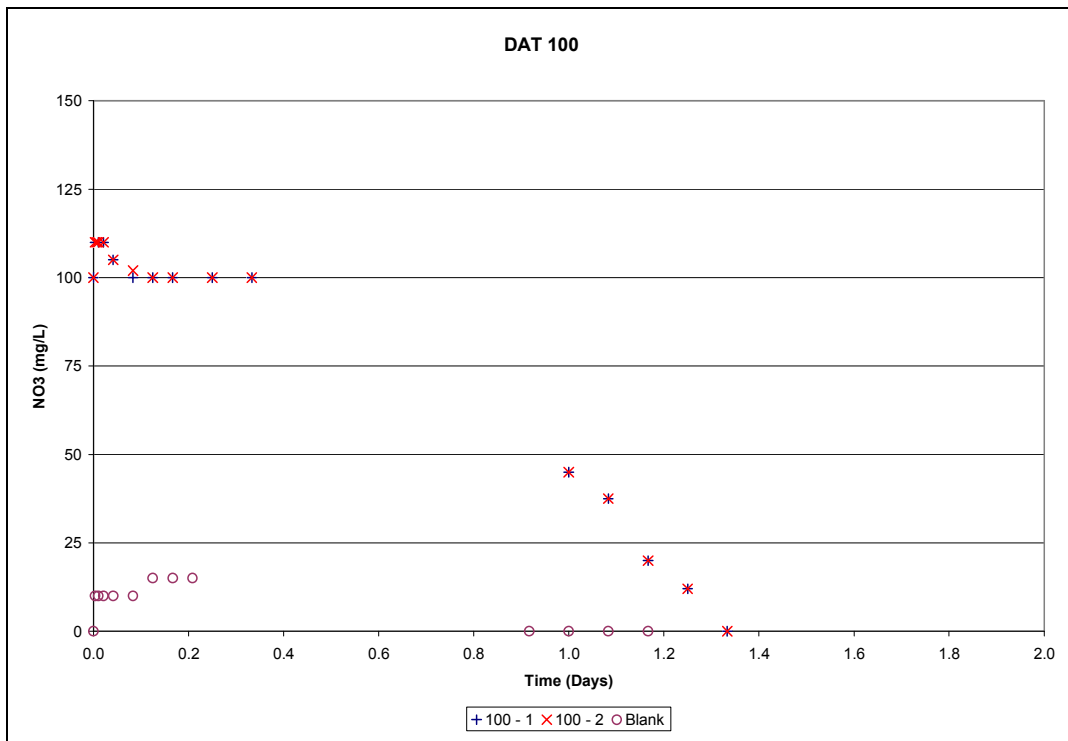
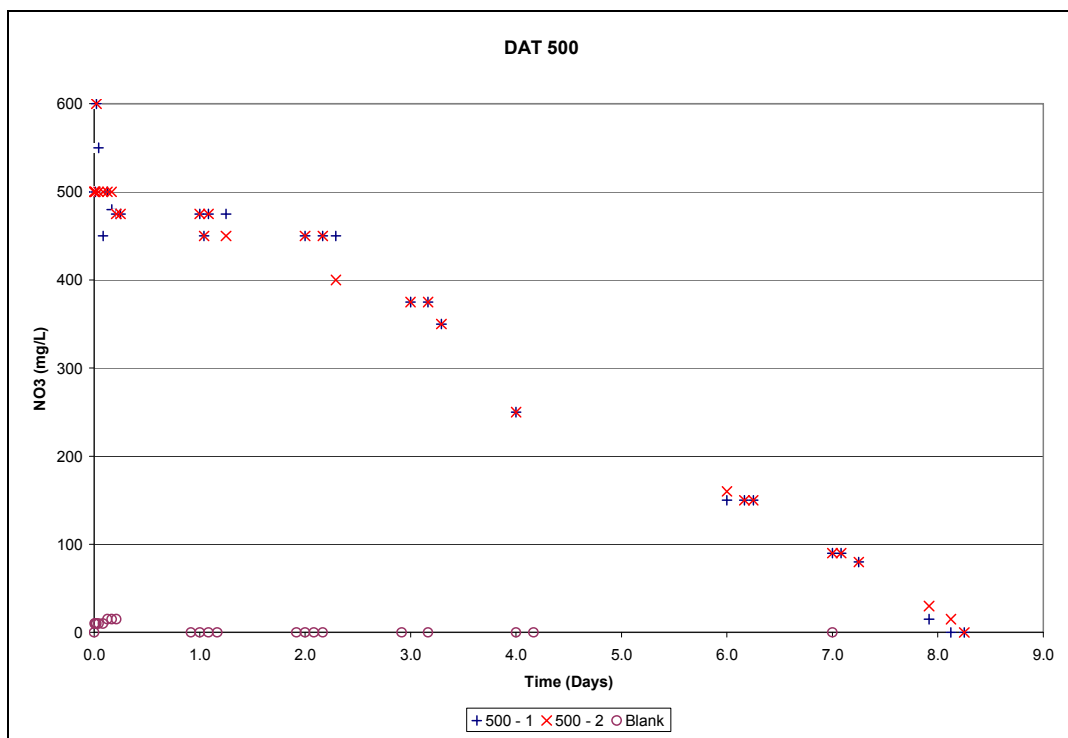
Table 4.12: Characterisation results of the input and output of the DAT batch tests.

Parameter	Input Eluate	Input Solid	Blank (0 mg/l)		100 (mg/l)		500 (mg/l)		2000 (mg/l)	
			Output Eluate	Output Solid	Output Eluate	Output Solid	Output Eluate	Output Solid	Output Eluate	Output Solid
pH	6.93		7.07		7.38		7.22		7.60	
COD (mg/l)	10080		8853		4165		7442		13712	
NH ₃ -N (mg/l)	29.04		7.0		4.3		28.0		14.3	
NO ₃ (mg/l)	8.96		0		0		0		0	
Total C (%)	0.60	22.04		40.5		25.5		35.3		13.9 – 23.1
Total N (%)	0.07	0.96		0.66		0.65		0.55		0.13 – 0.39
C/N Ratio	8.57	22.96		61.36		39.32		68.90		35.64 – 124.60

The pH remains constant around neutrality, while the COD results were all lower than the initial input value except in the case of the 2000 mg/l test. It is also noted that there was an increase in COD with an increase in the duration of each test, noting that the blank test was performed for the same duration as that of the 500 mg/l. NH₃ - N in the output values achieved in each test were lower than that of the input material.

The percentage of nitrogen decreased with the initial nitrate concentration. The C/N ratios for the tests were all higher than the input value.

The evolutions of the nitrate concentration for the tests conducted with the DAT substrate are shown in Figures 4.31, 4.32 and 4.33. The blank test showed little bioleaching but is included in each graph.

Figure 4.31: Evolution of the nitrate concentration for DAT at $C_0 = 100 \text{ mg/l}$ Figure 4.32: Evolution of the nitrate concentration for DAT at $C_0 = 500 \text{ mg/l}$

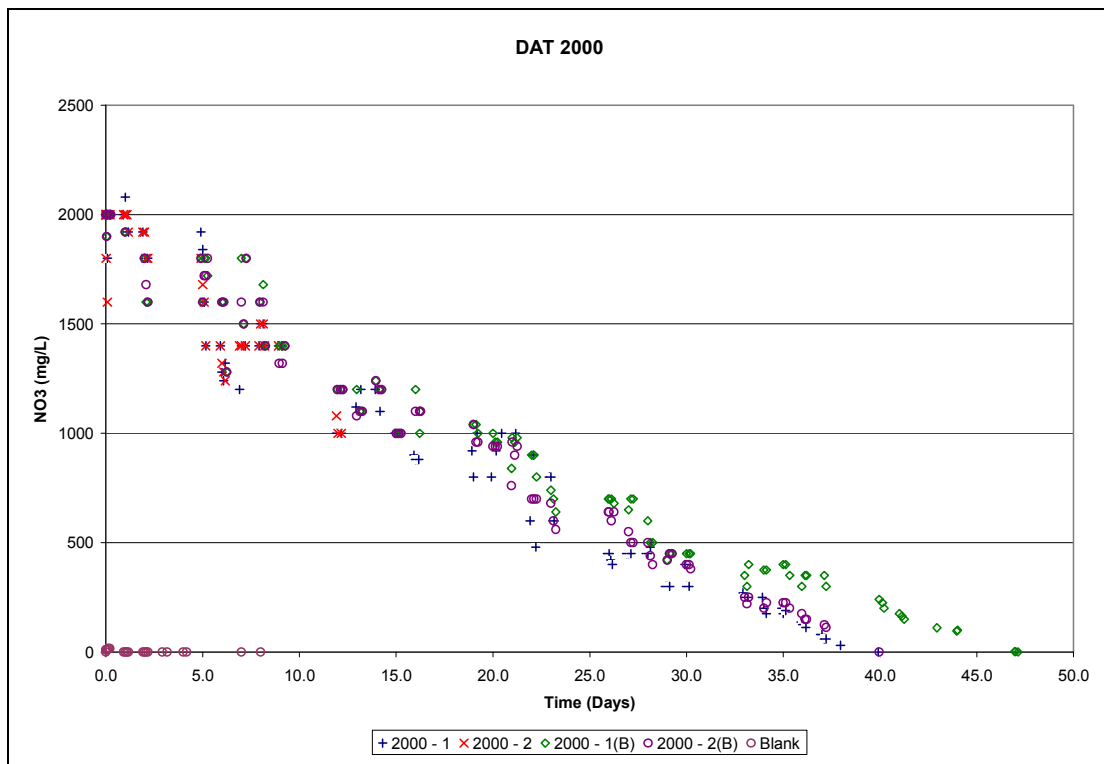


Figure 4.33: Evolution of the nitrate concentration for DAT at $C_0 = 2000 \text{ mg/l}$

Kinetics: Rate of Reaction

The results were modelled using a zero order kinetic reaction model.

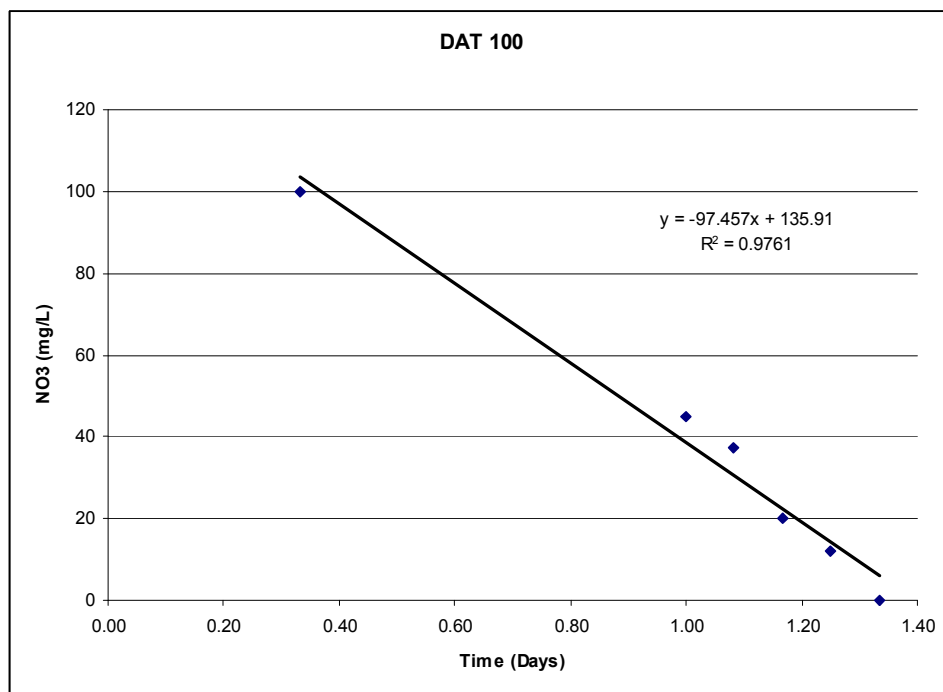
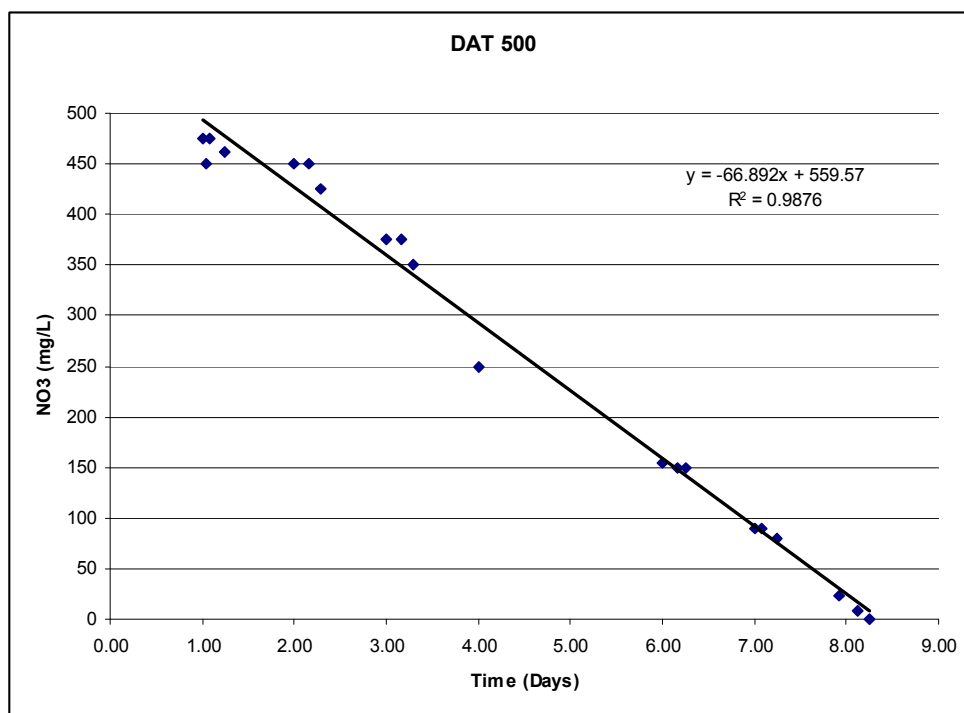
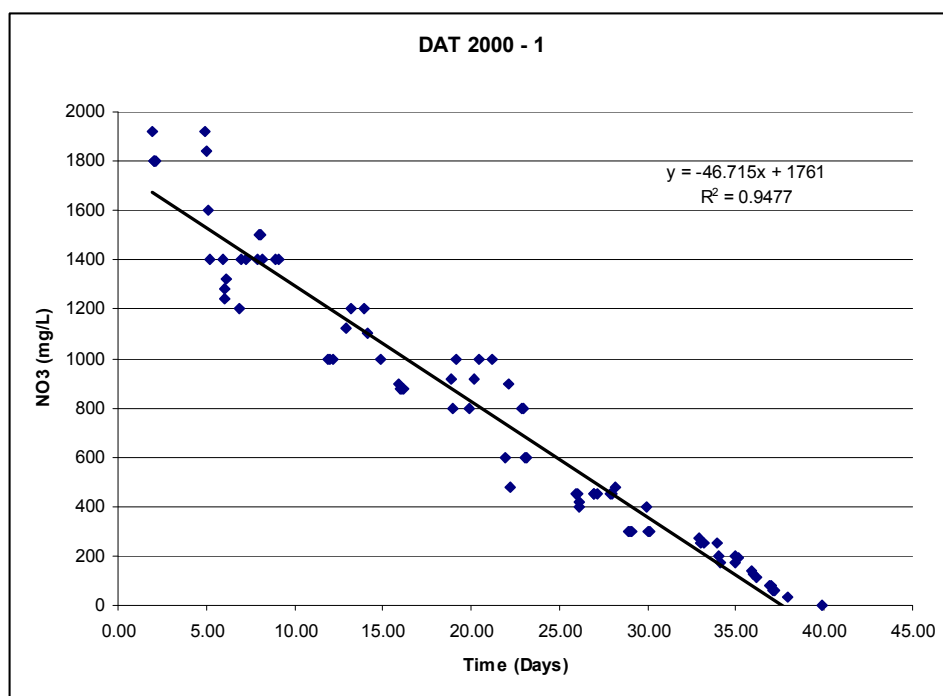


Figure 4.34: Kinetics of DAT at $C_0 = 100 \text{ mg/l}$

Figure 4.35: Kinetics of DAT at $C_o = 500 \text{ mg/l}$ Figure 4.36: Kinetics of DAT at $C_o = 2000 \text{ mg/l}$ Test 1

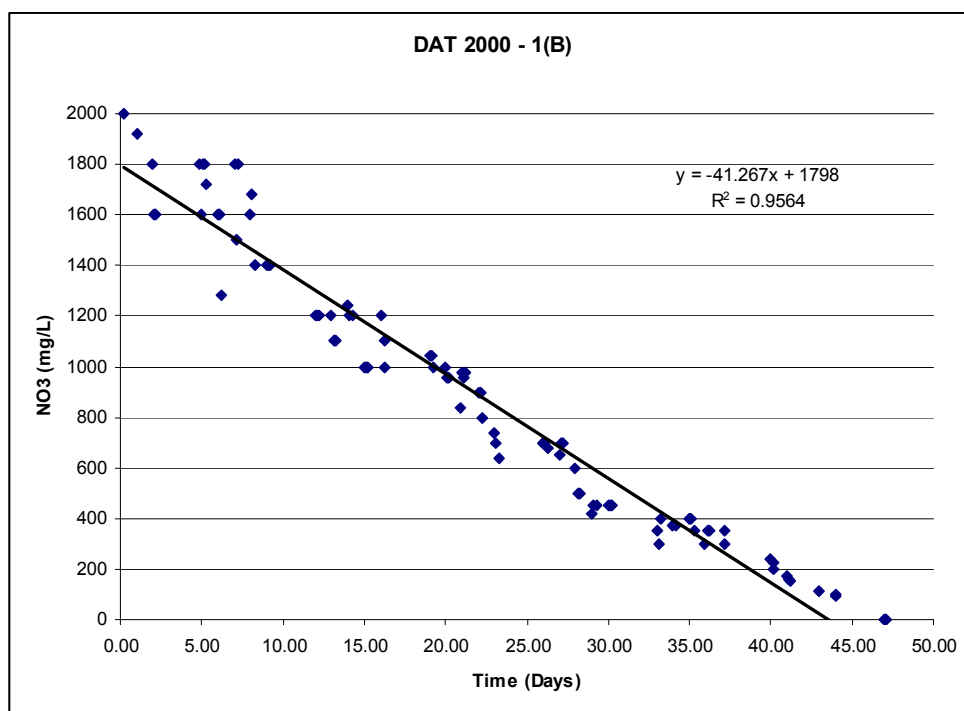
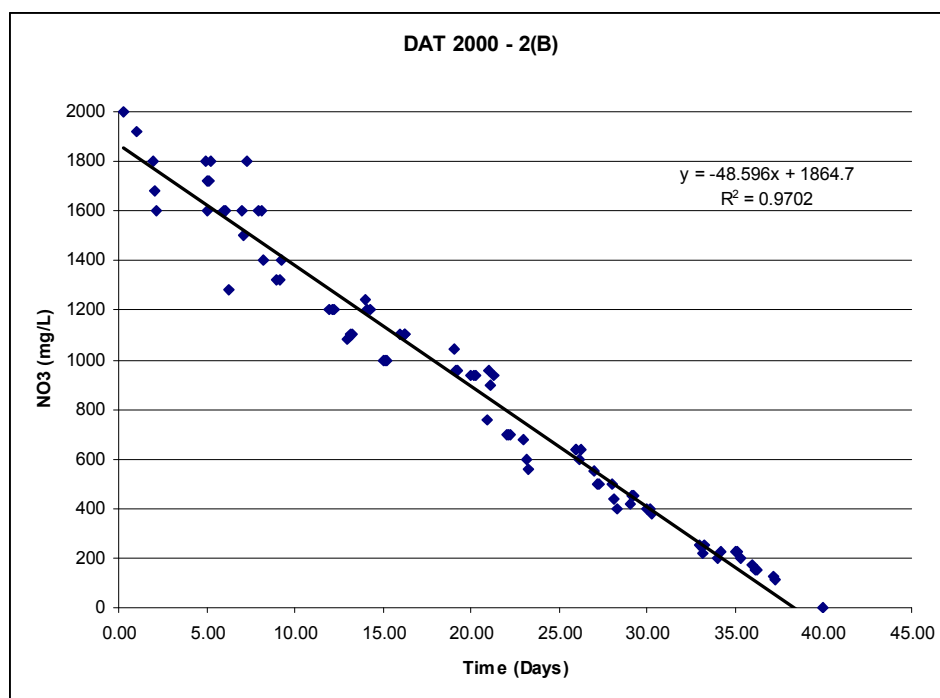
Figure 4.37: Kinetics of DAT at $C_0 = 2000 \text{ mg/l}$ Test 1(B)Figure 4.38: Kinetics of DAT at $C_0 = 2000 \text{ mg/l}$ Test 2(B)

Table 4.13 summarises the kinetic rates of removal over the linear period of each batch test.

Table 4.13: Summary of kinetics of DAT

C_o (mg/l)	Time for 100 % Removal (Days)	k (1/day)	R²	Percentage Removal (%)
100	1.33	97.46	0.98	100
500	8.25	66.89	0.99	100
2000 - 1	40.00	46.72	0.95	100
2000 – 1(B)	47.00	41.27	0.96	100
2000 – 2(B)	40.00	48.60	0.97	100

The three graphs of the respective tests all show a slight increase in the initial nitrate concentration at the beginning of each test. This could be due to the small percentage increase represented in the blank, due to an initial bioleaching of the organic nitrogen from the solid substrate. All tests also exhibit an initial plateau stage dependent on the nitrate concentration.

After acclimatisation, the curve displayed a linear relationship until full nitrate removal occurred in 1.3 days for the 100 mg/l test; 8 to 8.5 days for the 500 mg/l test and 40 days for the experiment at 2000 mg/l.

One of the 2000 mg/l tests took over 47 days to achieve full nitrate removal. This sample showed a considerably lower C/N ratio of 35.64 at the output of the test as compared to 100.43 and 120.60 in tests 2000 – 1 and 2000 – 2(B) respectively.

All the tests conducted with the DAT substrate achieved 100% nitrate removal, but more in depth investigations on the bio-denitrification patterns are required, as no microbial analysis was conducted on this substrate.

5. Mature Compost: Turned Windrow (TW)

Table 4.14 shows the characterisation results of the tests performed on the inputs and outputs in the batch tests (TW).

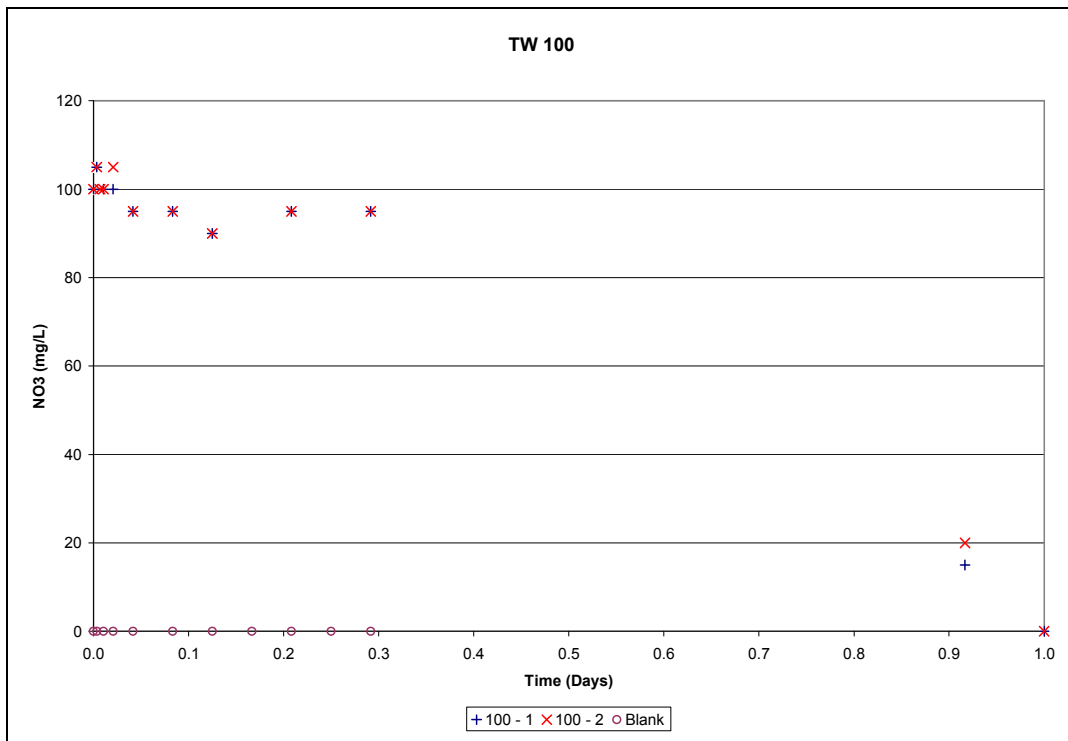
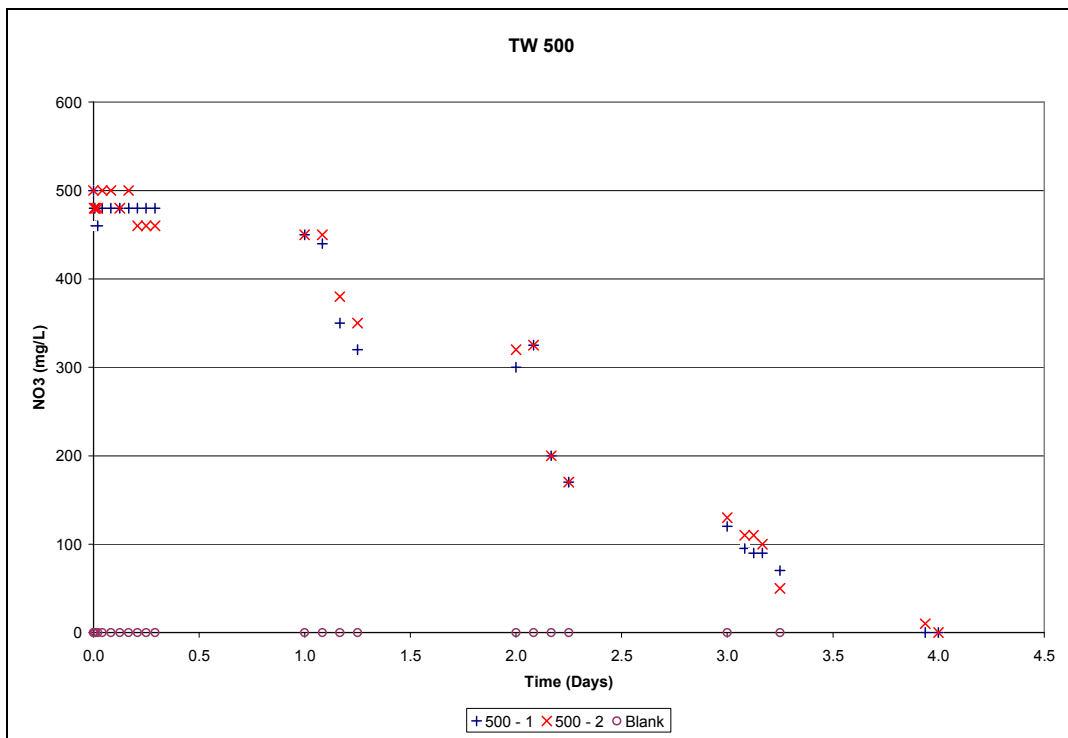
Table 4.14: Characterisation results of the input and output of the TW batch tests.

Parameter	Input Eluate	Input Solid	Blank (0 mg/l)		100 (mg/l)		500 (mg/l)		2000 (mg/l)	
			Output Eluate	Output Solid	Output Eluate	Output Solid	Output Eluate	Output Solid	Output Eluate	Output Solid
pH	7.27		7.13		7.86		7.58		7.51 – 7.88	
COD (mg/l)	11270		4570		4629		7396		7398 – 12359	
NH ₃ -N (mg/l)	50.12		5.0		2.3		12.0		7.5 – 10	
NO ₃ (mg/l)	14.46		0		0		0		0	
Total C (%)	0.67	29.04		40.0		30.8		46.9		31.2 – 41.8
Total N (%)	0.09	1.65		1.62		1.73		1.68		0.82 – 1.53
C/N Ratio	7.44	17.60		24.69		19.23		28.05		26.33 – 47.07

Table 4.14 shows a similar trend observed for the DAT substrate.

The evolution of the nitrate concentration for the TW substrate conducted for each of the initial concentrations is shown in Figures 4.39, 4.40 and 4.41.

The blank test showed no leaching out of nitrates; however its results are still included in each of the graphs.

Figure 4.39: Evolution of the nitrate concentration for TW at $C_0 = 100 \text{ mg/l}$ Figure 4.40: Evolution of the nitrate concentration for TW at $C_0 = 500 \text{ mg/l}$

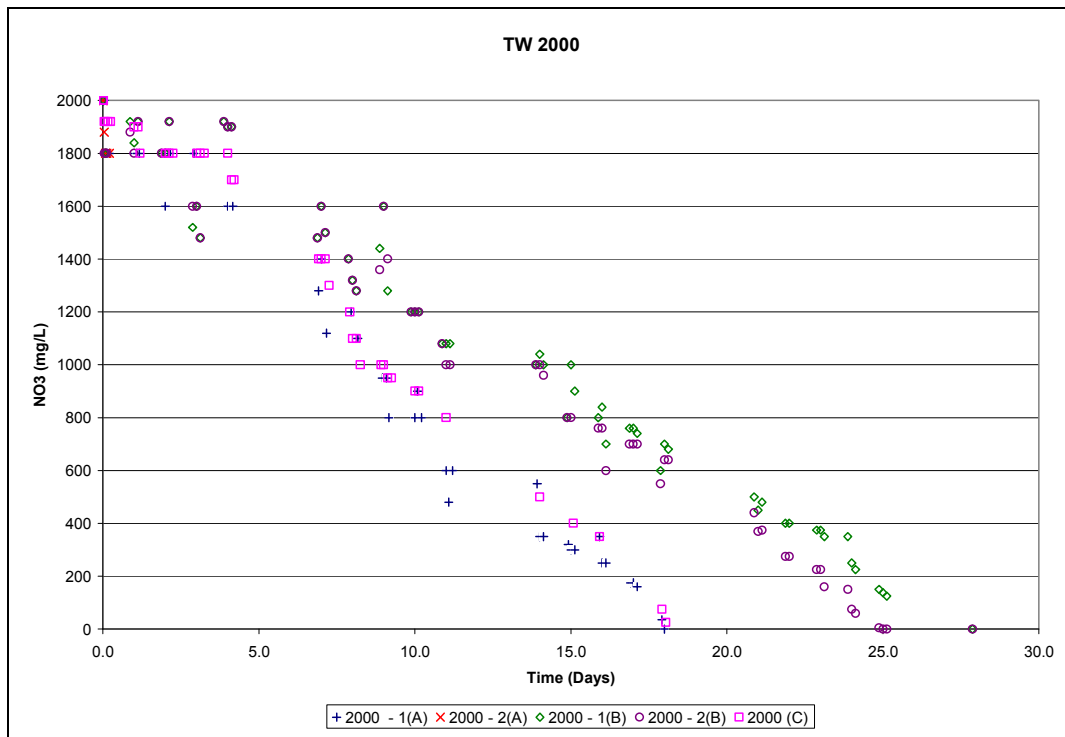


Figure 4.41: Evolution of the nitrate concentration for TW at $C_0 = 2000 \text{ mg/l}$

Kinetics: Rate of Reaction

The results were modelled using a zero order kinetic reaction model.

Rate of Reaction for linear period:

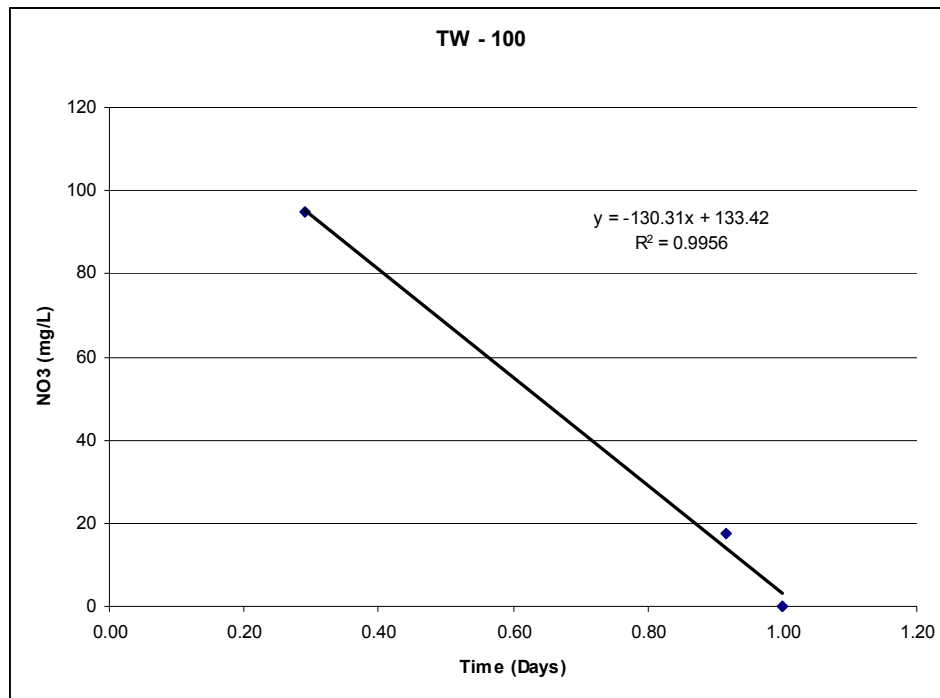


Figure 4.42: Kinetics of TW at $C_0 = 100 \text{ mg/l}$

In Figure 4.42, only three points were used to model the kinetic reactions, due to the duration of the plateau phase which enforced the test to be continued over night resulting in the time gap from 0.3 – 0.95 days.

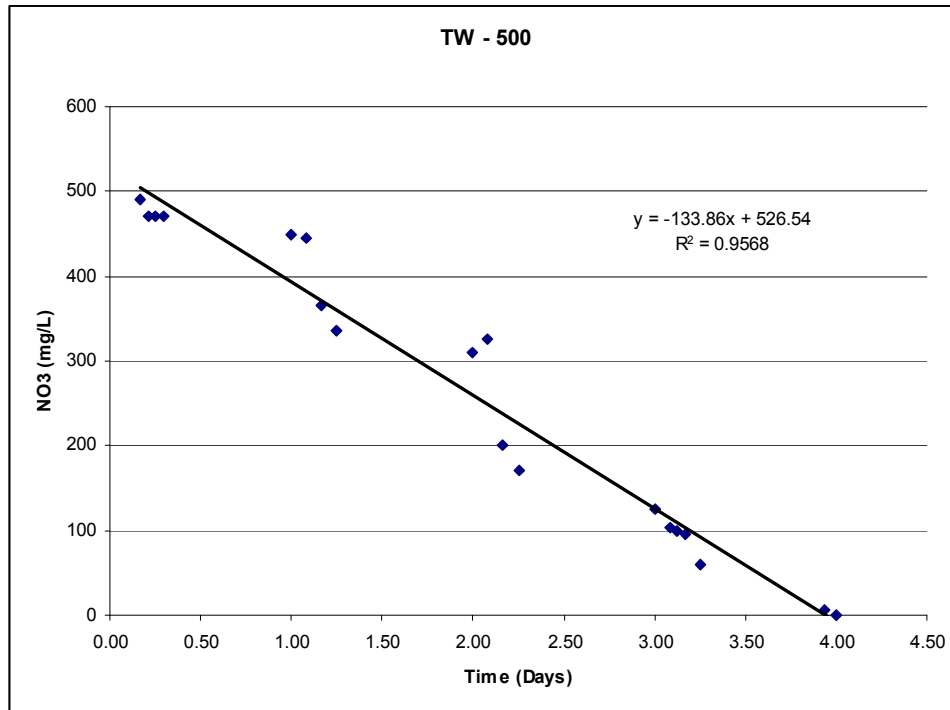


Figure 4.43: Kinetics of TW at $C_0 = 500 \text{ mg/}\ell$

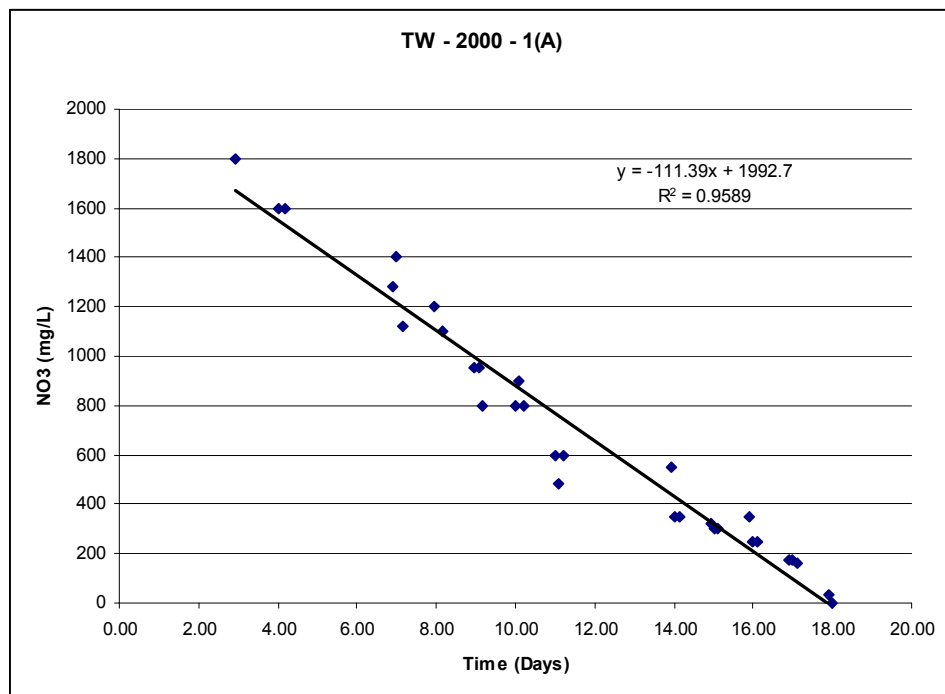
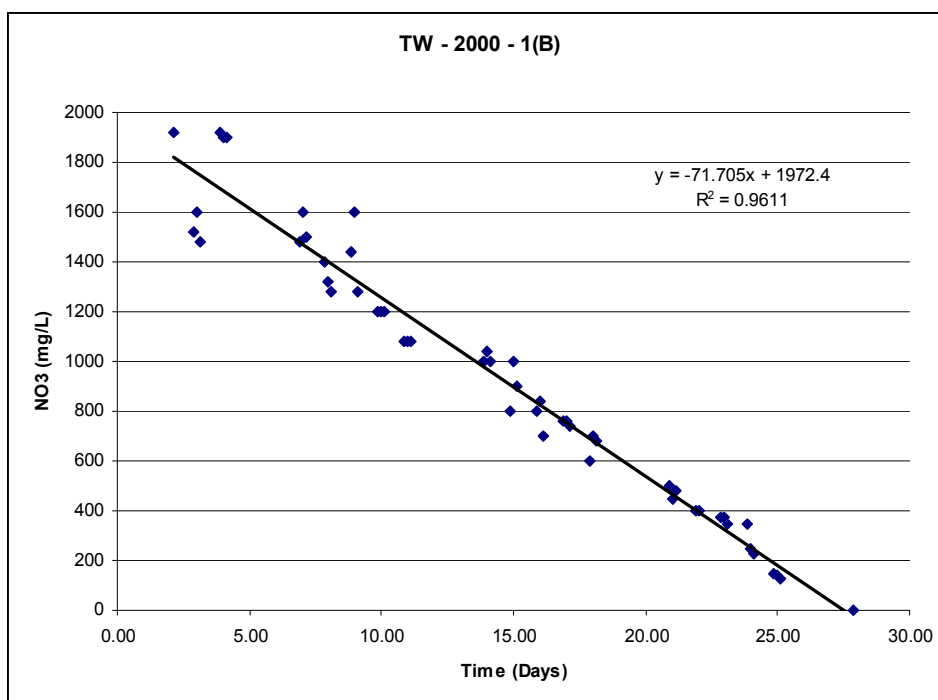
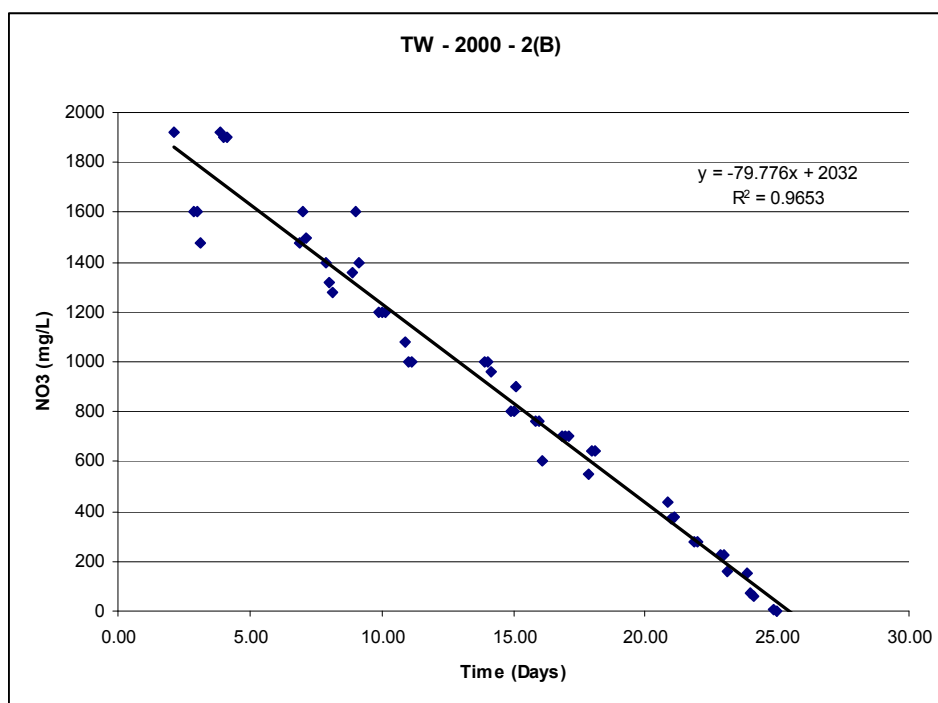


Figure 4.44: Kinetics of TW at $C_0 = 2000 \text{ mg/}\ell$ Test 1(A)

Figure 4.45: Kinetics of TW at $C_0 = 2000 \text{ mg/l}$ Test 1(B)Figure 4.46: Kinetics of TW at $C_0 = 2000 \text{ mg/l}$ Test 2(B)

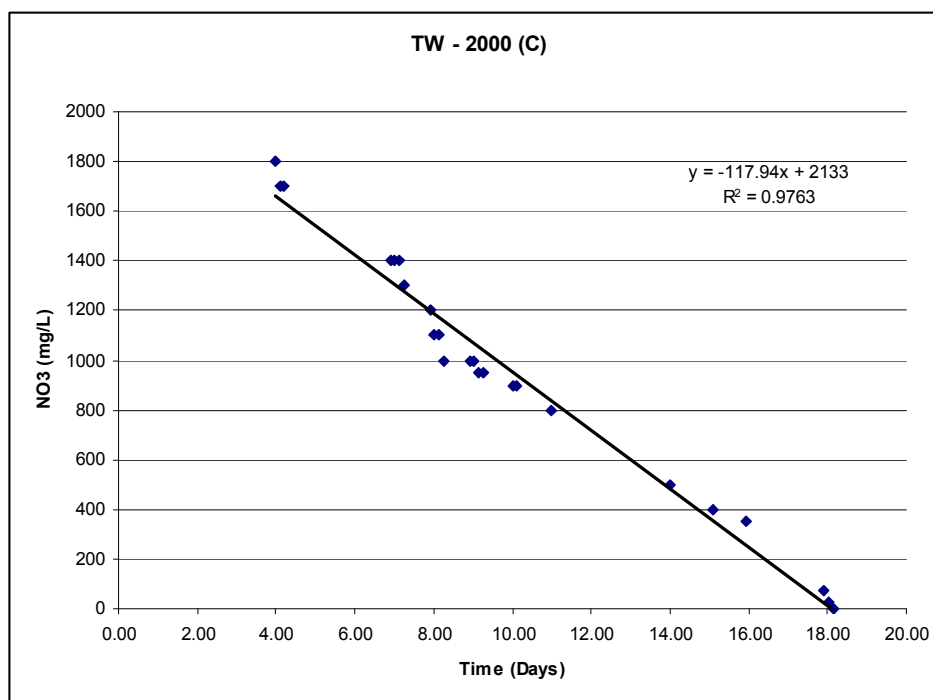
Figure 4.47: Kinetics of TW at $C_0 = 2000 \text{ mg/l}$ Test (C)

Table 4.15 summarises the kinetic rates of removal over the linear period of each batch test.

Table 4.15: Summary of kinetics of TW

C_0 (mg/l)	Time for 100% Removal (Days)	k (1/day)	R^2	Percentage Removal (%)
100	1	130.31	0.996	100
500	4	133.86	0.96	100
2000 – 1[A]	18	111.39	0.96	100
2000 – 1[B]	27	71.71	0.96	100
2000 – 2[B]	25	79.78	0.97	100
2000 [C]	18	117.94	0.98	100

All the TW tests initially displayed an expected plateau stage that lasted approximately 0.3 days for the 100 mg/l test, 1 day for the test at $C_0 = 500 \text{ mg/l}$ and 4 days for the test at $C_0 = 2000 \text{ mg/l}$. After the plateau stage, once each test had reached its regime, denitrification occurred at a linear rate until the final nitrate concentration level reached zero. The 100 mg/l test reached full nitrate removal after 1 day. The 500 mg/l took longer and achieved full nitrate removal after 4 days.

As seen from Figure 4.41, four tests were conducted for the initial nitrate concentration of 2000 mg/l. These tests were not all conducted at the same time as represented by the graph labels. The four tests presented two different behaviours. One pair of tests reached full nitrate removal after 18 days where as the other pair took more than a week longer and finished between 25 and 27 days.

As noted for the DAT substrate, the test at 100 mg/l took less than the 74 hours to achieve full nitrate removal, and it is, therefore, uncertain whether nitrate removal occurred through bio-denitrification or other mechanisms.

4.4. Column Tests

4.4.1. Substrate Selection

The following criteria were used to determine which substrates were to be utilised in the column studies. The first key parameter was the C/N ratio of the substrate. It is essential to have a relatively high C/N ratio for denitrification. C/N ratios above 16 were considered suitable for denitrification (Tsui et al., 2007; Wu et al., 2001; Trois et al., 2010). The second parameter was the pH. The optimum range of pH for denitrification is 6 – 8. The third parameter used for assessing the suitability of a substrate was the time required for full denitrification to be achieved in optimum conditions, as achieved in the batch tests. The capacity of the substrates to release COD and NH_3 through bioleaching was also taken into account.

A summary of the substrates and the criteria used for their utilisation in the column studies are shown in Table 4.16 for nitrate concentrations of 500 and 2000 mg/l.

Table 4.16: Summary of column test criteria at $C_0 = 500$ and 2000 mg/l

Substrate	Input		COD (mg/l)		Time for 100% Removal (Days)	
	C/N Ratio	pH	500	2000	500	2000
Pine Bark	62.15	4.18	14157	13245	-	-
CGR RAW	90.19	5.45	3951 - 7200	7009 – 7870	0.5 or 7.83	10.5
DGR 10	12.75	7.40	17783		9.5	34.5
CGR 10	23.91	6.98	3177		8	22
DAT	22.96	6.93	7442	13712	8.25	40 – 47
TW	17.60	7.27	7396	7398 - 12359	4	18 - 27

The substrates chosen were the fresh pine bark (PB), the fresh CGR (CGR RAW) and the immature CGR compost (CGR 10).

CGR RAW was chosen due to its high C/N ratio, relatively low COD output and best performance in the batch tests. Pine Bark was chosen because it displays the second highest C/N ratio, positive results in column testing in previous studies (Trois et al., 2010; Diaz et al., 2003; Pisano, 2007). CGR 10 was selected as it has the third highest C/N ratio, a suitable pH in the optimum range and a lower output COD value.

DAT and DGR 10 were excluded due to their slower rate of removal and high COD released at all initial nitrate concentrations.

A summary of column operating conditions is presented in Table 4.17. The raw data recorded for each experiment can be seen in Appendix C.

Table 4.17: Summary of column operating conditions

Column	Substrate	NO ₃ Concentration (mg/l)	Duration (Weeks)	Flow Rates (l/day)	
				Experiment 1	Experiment 2
1	CGR RAW	500	4	2.48	5.625
2	PB	500	4	2.00	5.00
3	CGR 10	500	4	1.7	2.85
4	CGR RAW	2000	4	2.38	5.65
5	PB	2000	4	2.00	5.00
6	CGR 10	2000	4	1.78	2.85

4.4.2. Experiment 1

The flow of nitrate solution replacement through a typical column and the days spent in contact with the substrate over a two week period are shown in Figure 4.48.

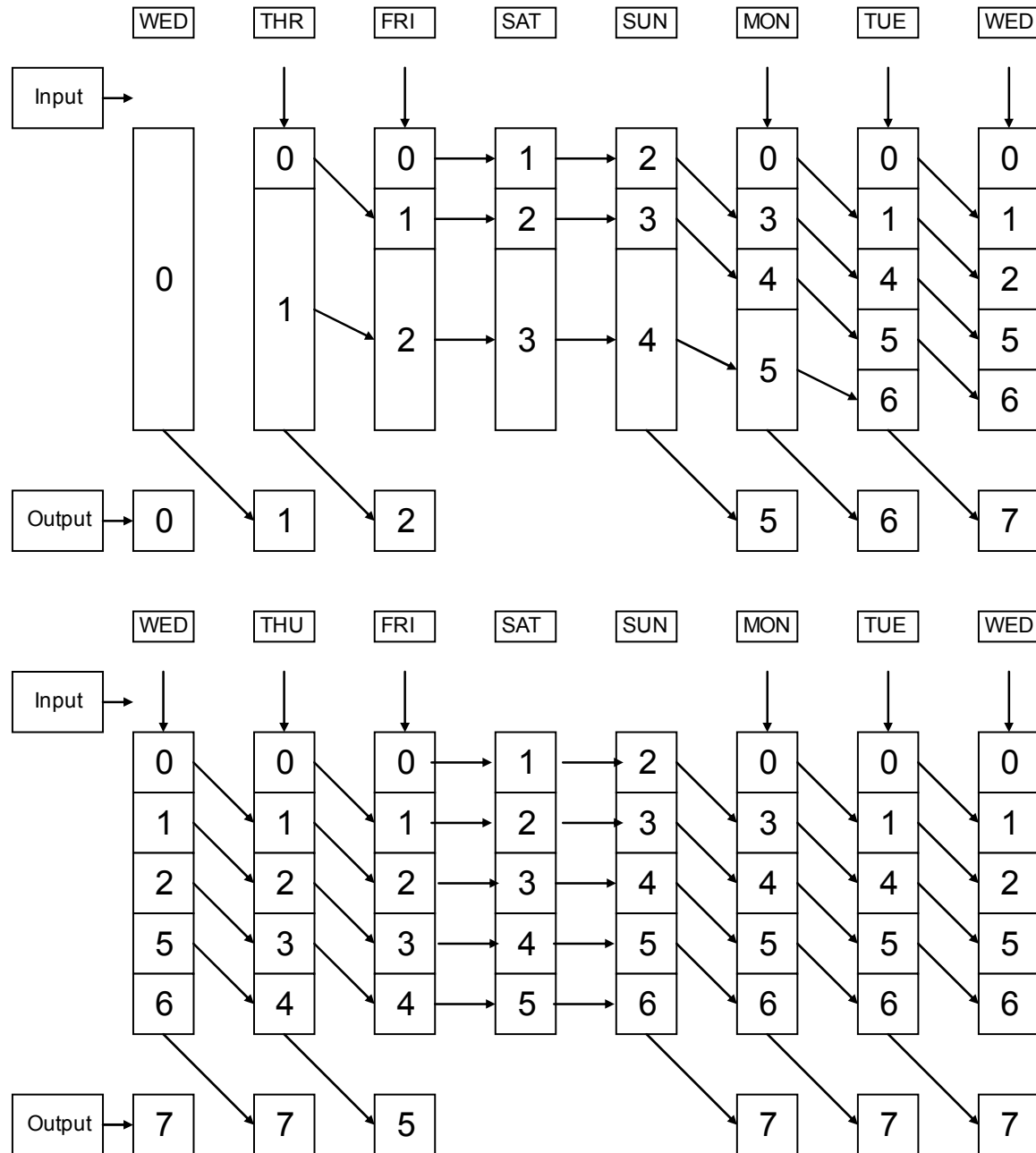


Figure 4.48: Experiment 1 – Nitrate solution replacement flow diagram

4.4.3. Experiment 2

The flow of nitrate solution replacement through a typical column and the days spent in contact with the substrate over a two week period are shown in Figure 4.49.

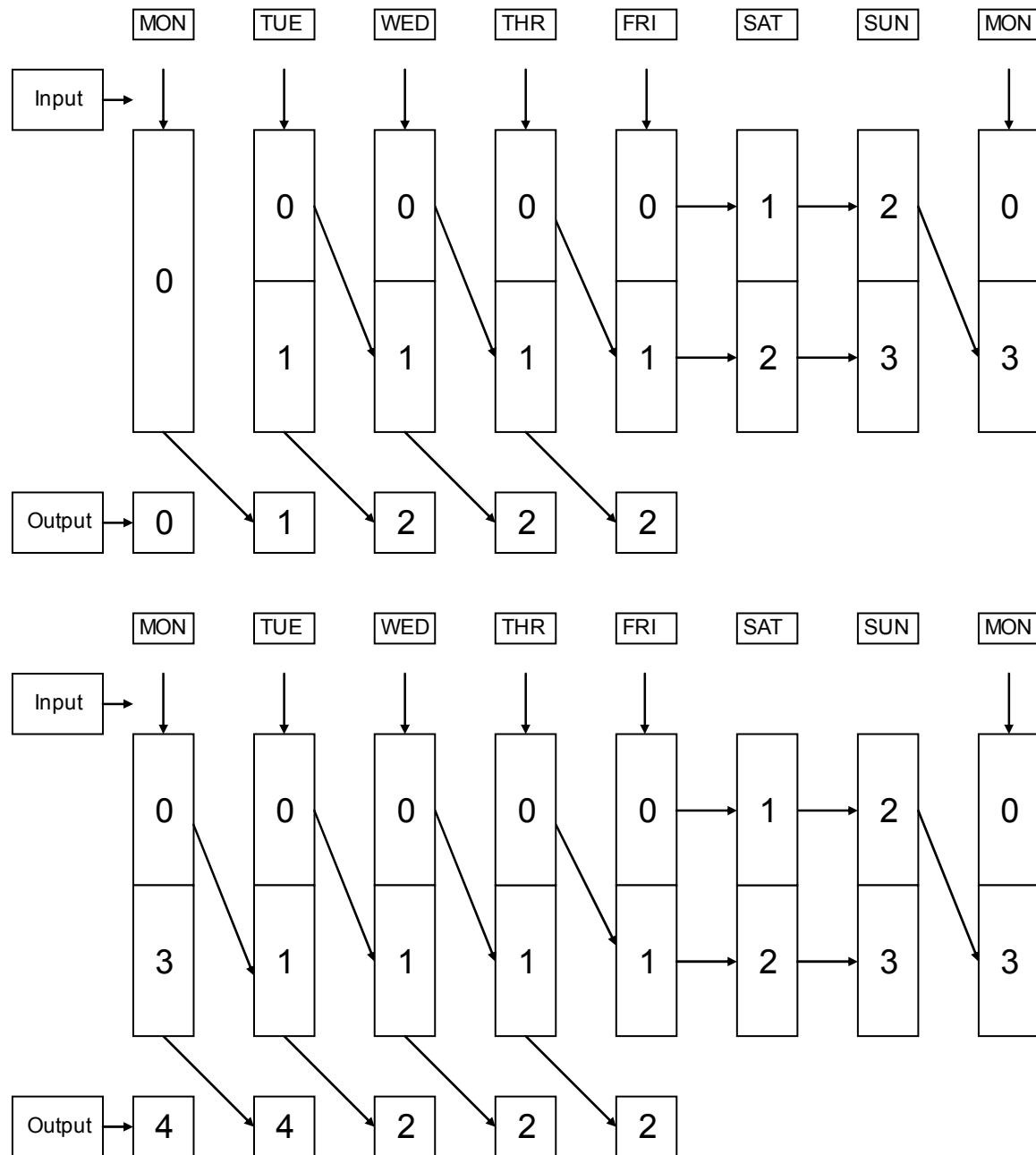


Figure 4.49: Experiment 2 - Nitrate solution replacement flow diagram

4.4.4.1. Fresh CGR (CGR RAW)

$C_o = 500 \text{ mg/}\ell$

The evolution of the nitrate concentrations and pH over the two flow rates for the CGR RAW substrate are shown in Figures 4.50. and 4.51.

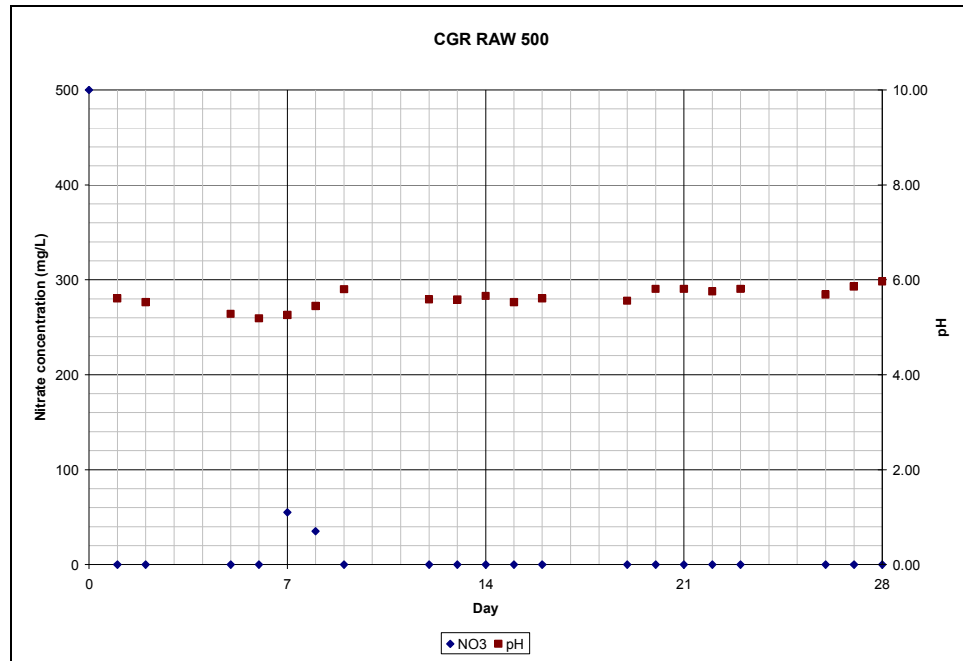


Figure 4.50: Experiment 1 - Evolution of the nitrate concentration and pH for CGR RAW for $C_o = 500 \text{ mg/}\ell$ at flow rate 1

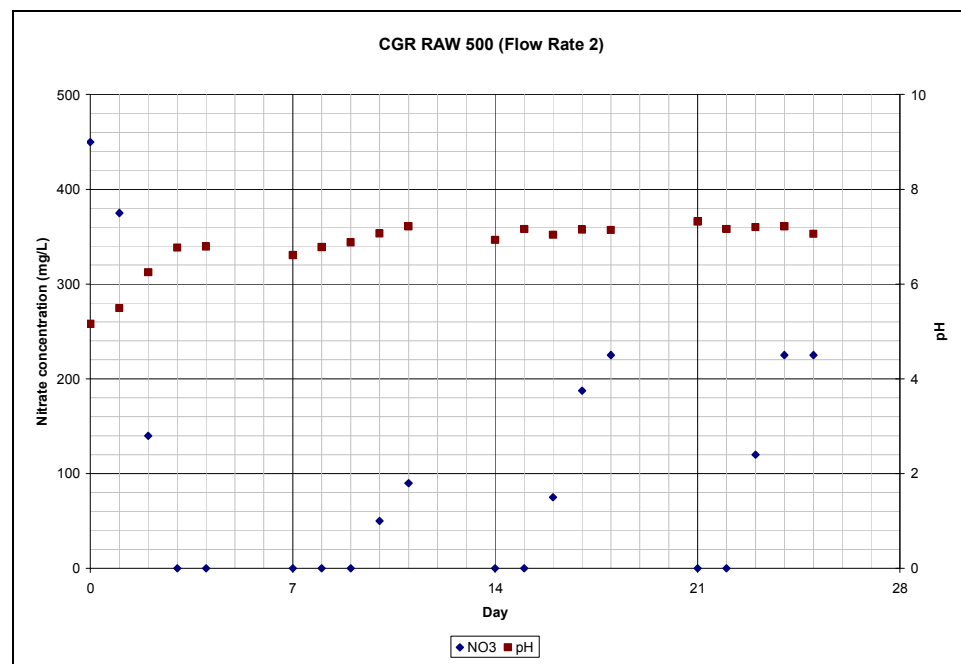


Figure 4.51: Experiment 2 - Evolution of the nitrate concentration and pH for CGR RAW for $C_o = 500 \text{ mg/}\ell$ at flow rate 2

The evolution of the nitrate concentration over the length of the column for flow rate 1 is shown in Figure 4.52. The graph demonstrates the Nitrate Concentration (NO_3) in mg/ℓ in relation to length recorded in metres.

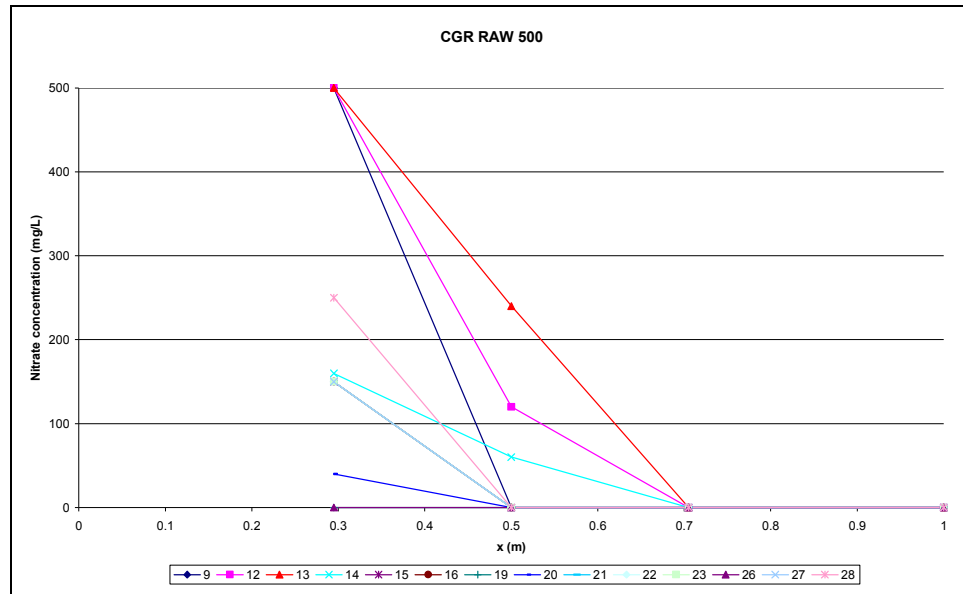


Figure 4.52: Experiment 1 - Evolution of the nitrate concentration over the column length for CGR RAW for $C_0 = 500 \text{ mg}/\ell$ at flow rate 1

The COD of the output for the CGR RAW substrate at 500 mg/l are shown in Figures 4.53. and 4.54.

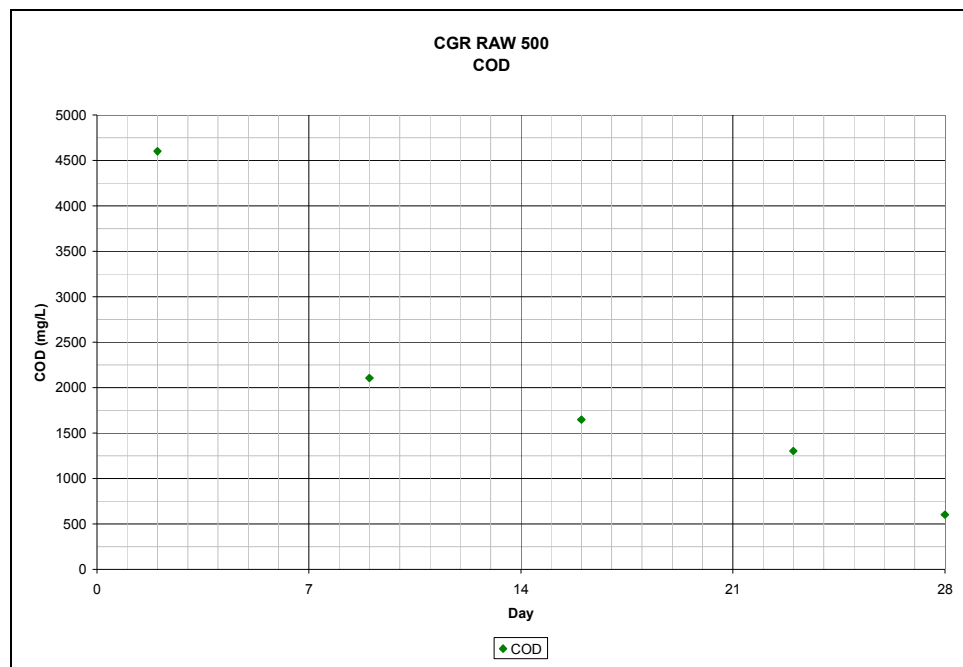


Figure 4.53: Experiment 1 – Evolution of COD for CGR RAW for $C_o = 500 \text{ mg/l}$ at flow rate 1

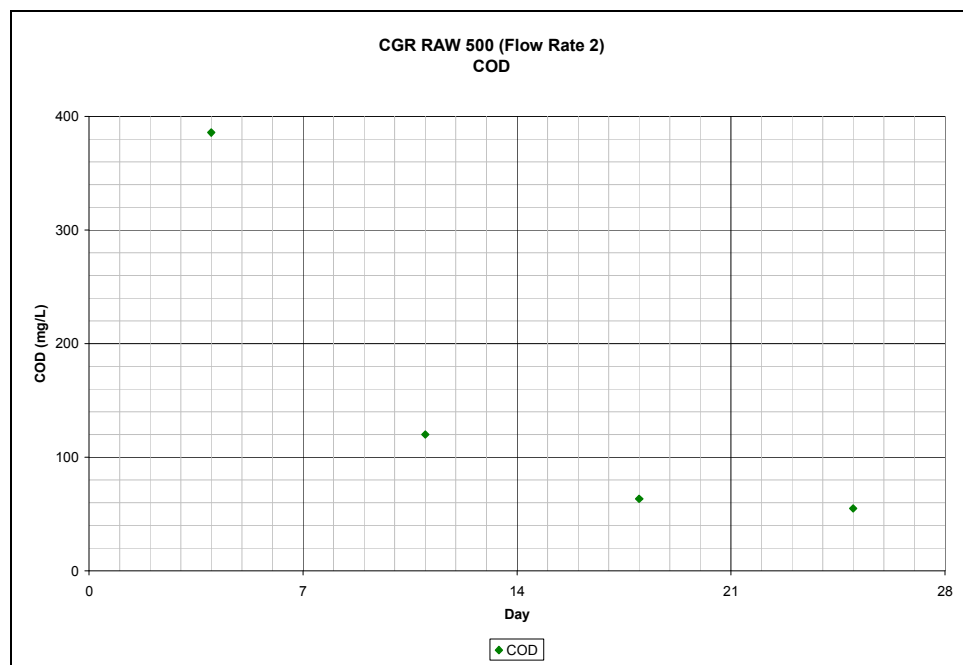


Figure 4.54: Experiment 2 – Evolution of COD for CGR RAW for $C_o = 500 \text{ mg/l}$ at flow rate 2

Full nitrate removal was achieved within the first 5 days at flow rate 1 and initial 4 days at flow rate 2. For the latter, there was insufficient contact time between the solution and the substrate during weeks 2, 3 and 4, causing a rise in nitrate concentration. However after the extended contact time over the weekend, the entire column had achieved full nitrate removal.

The COD of the output effluent dropped considerably throughout the period of the test. After the first week a value of above 4500 mg/l was recorded, however the COD dropped by more than 85% by the end of the experiment 1. The COD results at the second flow rate are lower than those recorded in experiment 1. This is due to the fact that the substrate was not replaced over the two experiments. Experiment 2 displayed a drop of 88%, with a final output of 55 mg/l.

The pH remained below 6 during experiment 1 and tended to rise during the first week to 7 and remained at this level throughout the rest of experiment 2. The temperature remained constant with a range between 19 and 22 °C, whilst the determined $\text{NH}_3 - \text{N}$ dropped to less than 1 mg/l at the conclusion of both experiments.

$C_o = 2000 \text{ mg/}\ell$

The evolution of the nitrate concentrations and pH over the two flow rates for the CGR RAW substrate are shown in Figures 4.55 and 4.56.

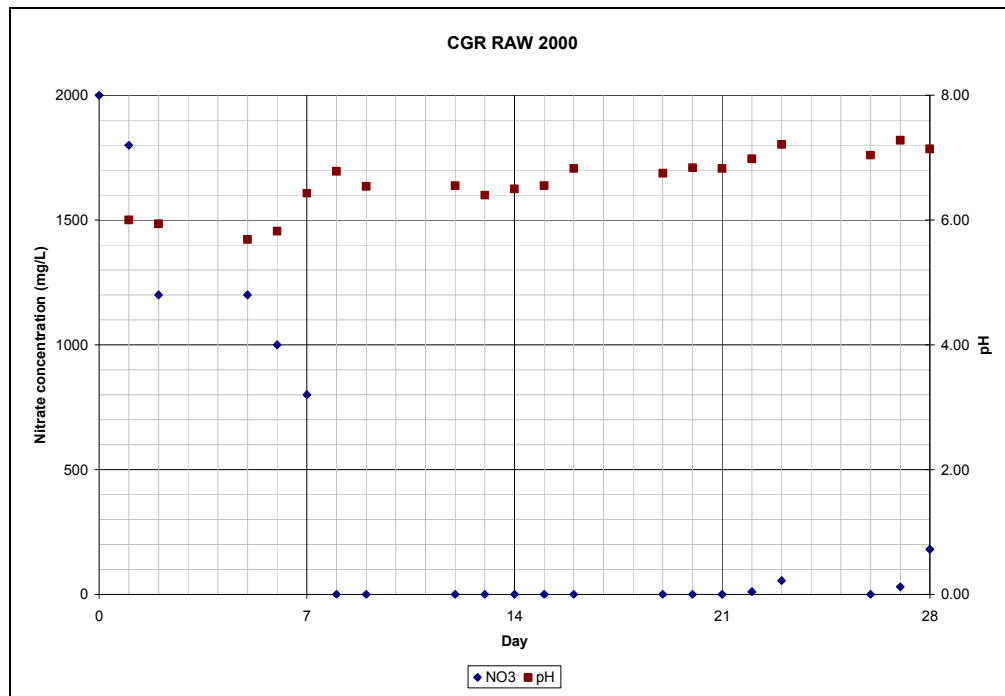


Figure 4.55: Experiment 1 - Evolution of the nitrate concentration and pH for CGR RAW for $C_o = 2000 \text{ mg/}\ell$ at flow rate 1

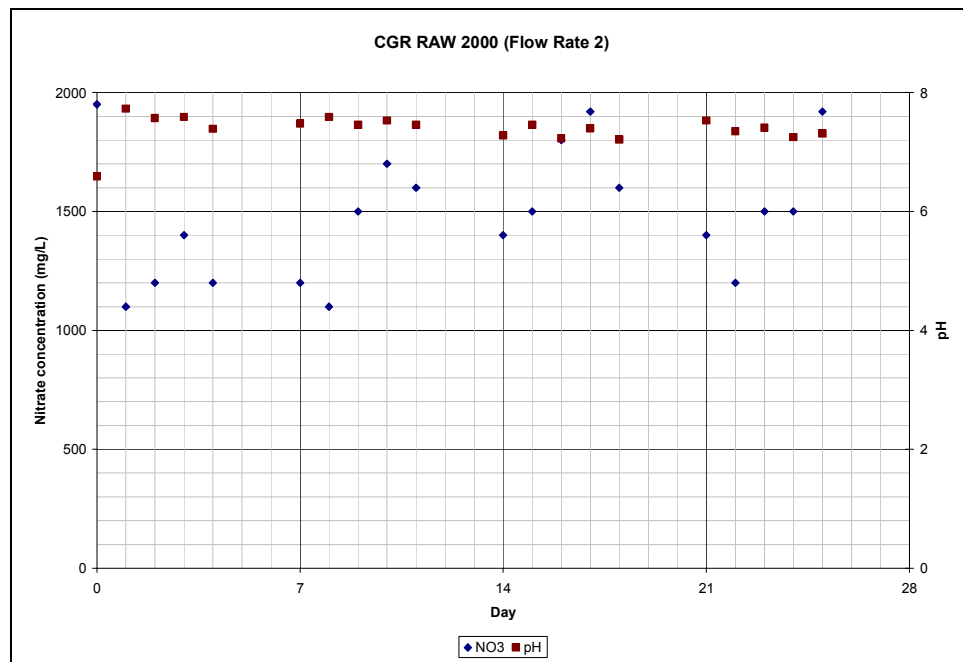


Figure 4.56: Experiment 2 - Evolution of the nitrate concentration and pH for CGR RAW for $C_o = 2000 \text{ mg/}\ell$ at flow rate 2

The evolution of the nitrate concentration over the length of the column for flow rate 1 is shown in Figure 4.57.

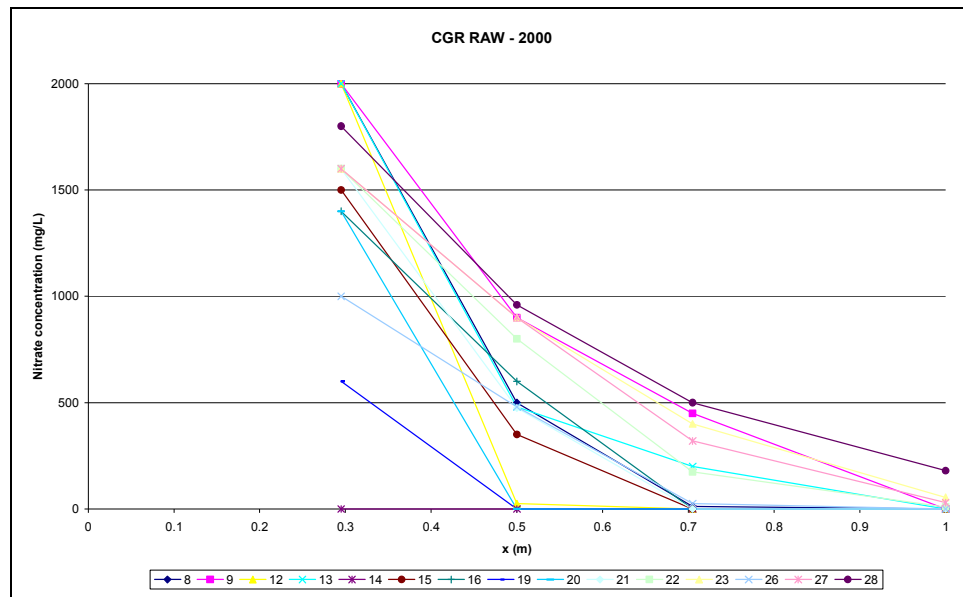


Figure 4.57: Experiment 1 - Evolution of the nitrate concentration over the column length for CGR RAW for $C_0 = 2000 \text{ mg/l}$ at flow rate 1

The COD of the output for the CGR RAW substrate at 2000 mg/l are shown in Figures 4.58 and 4.59.

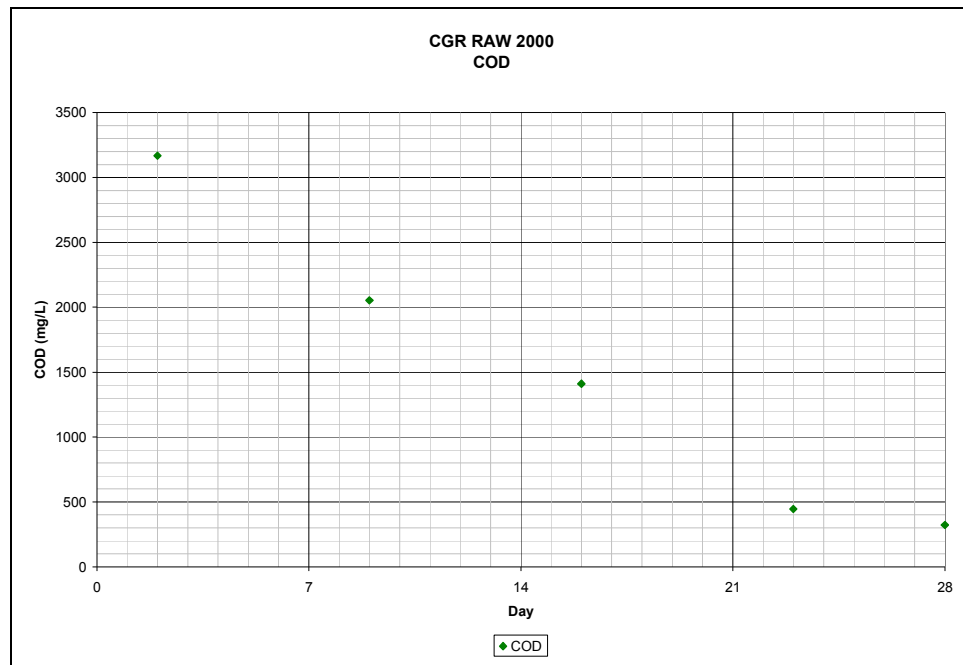


Figure 4.58: Experiment 1 – Evolution of COD for CGR RAW for $C_0 = 2000 \text{ mg/l}$ at flow rate 1

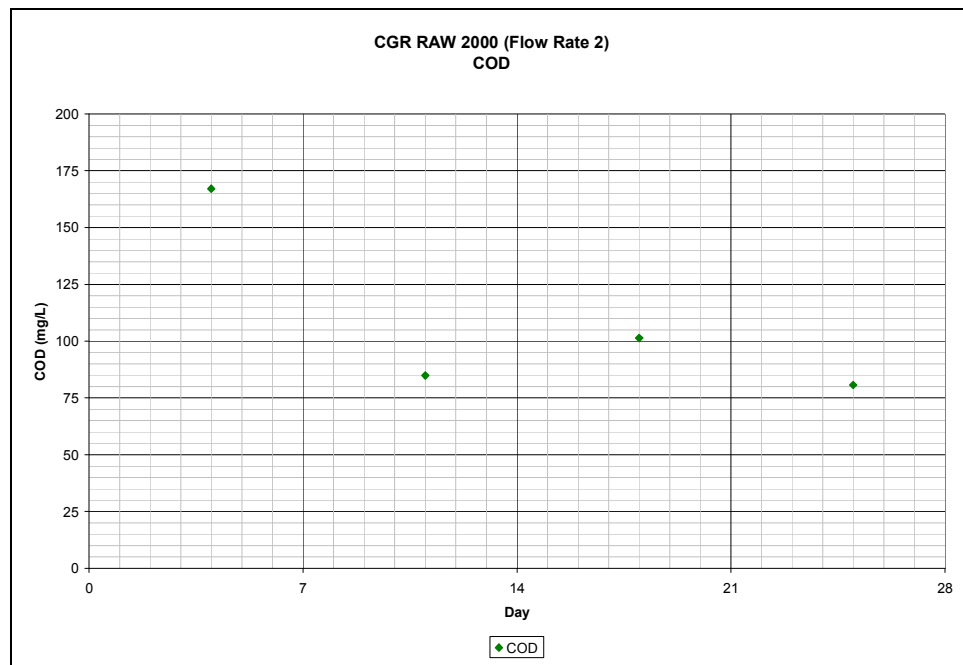


Figure 4.59: Experiment 2 – Evolution of COD for CGR RAW for $C_0 = 2000 \text{ mg/l}$ at flow rate 2

The nitrate concentration in the column at flow rate 1 reached zero after the initial 7 days. The concentration at the bottom of the column remained at zero until day 22, where the output concentration rose. This was observed once again during the following week. This reduced rate of denitrification could be due to the high nitrate concentration saturating the substrate. The rate at which carbon was being released had reduced and was now slower than the rate at which nitrates were being added. During the second week, full nitrate removal was being achieved within 1 - 2 days. However as the experiment progressed, this rate of denitrification reduced. At the end of the period the substrate failed to fully denitrify the leachate.

At flow rate 2, the coupled effect of the very high nitrate concentration and high flow rate negatively affected the performance of the test resulting in a lower denitrification rate and only 50% removal efficiency against 100% in the first experiment.

The COD of the output effluent dropped considerably through the period of the test 1 at a constant rate, from 3200 mg/l to 400 mg/l, with 88% removal. However, the COD values during experiment 2 dropped after the first week to below 100 mg/l where it remained fairly constant throughout the duration of the experiment. At the end of the experiment the final COD value was below 100 mg/l.

The pH during experiment 1 tended to increase to neutrality, whilst the pH during the experiment 2 stayed constant between 7 and 8 after an initial rise from 6.79 on the first day. The temperature remained constant with a range between 19 and 23 °C. In experiment 1, the $\text{NH}_3 - \text{N}$ was 14 to 16 mg/l over the first two weeks and dropped to below 5mg/l for the remaining weeks of the experiment. The measured $\text{NH}_3 - \text{N}$ during experiment 2 remained fairly constant with a range between 1.5 and 7.0 mg/l.

4.4.4.2. Fresh Pine bark (PB)

$C_o = 500 \text{ mg/l}$

The evolution of the nitrate concentrations and pH over the two flow rates for the Pine bark substrate are shown in Figures 4.60 and 4.61.

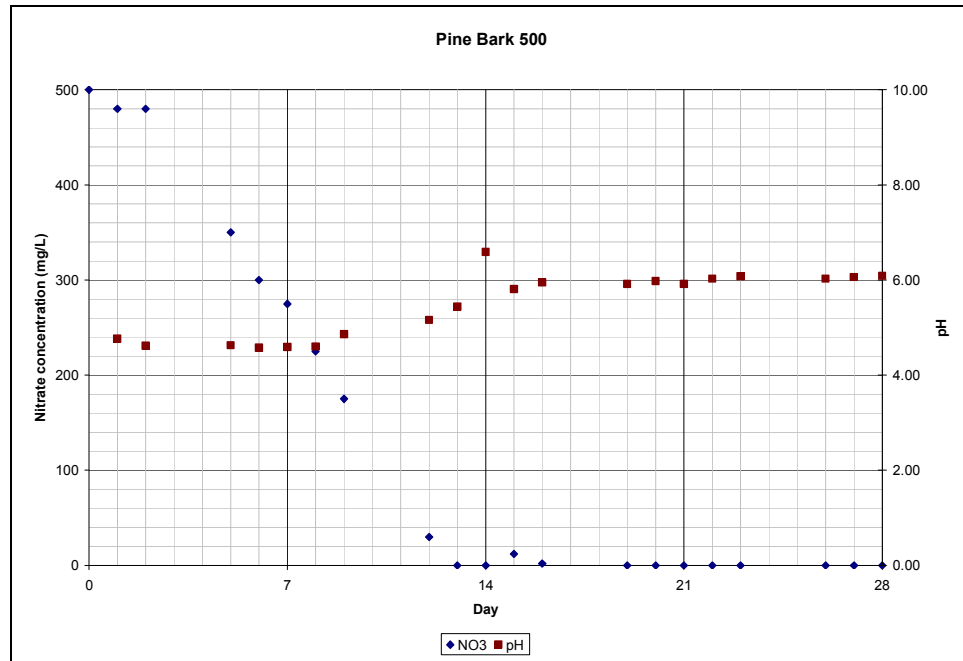


Figure 4.60: Experiment 1 - Evolution of the nitrate concentration and pH for PB for $C_o = 500 \text{ mg/l}$ at flow rate 1

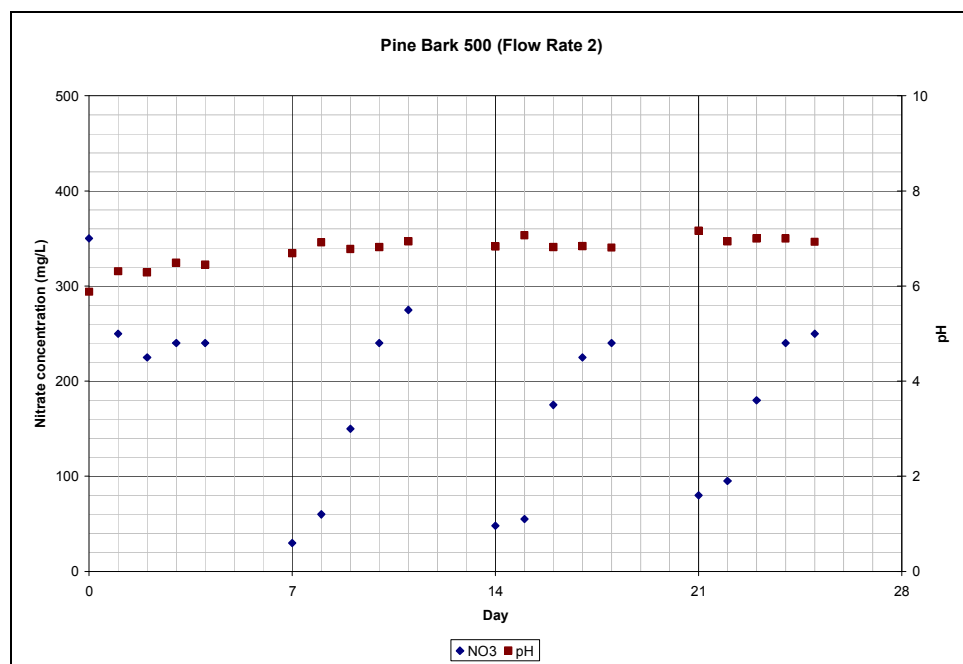


Figure 4.61: Experiment 2 - Evolution of the nitrate concentration and pH for PB for $C_o = 500 \text{ mg/l}$ at flow rate 2

The evolution of the nitrate concentration over the length of the column for flow rate 1 is shown in Figure 4.62.

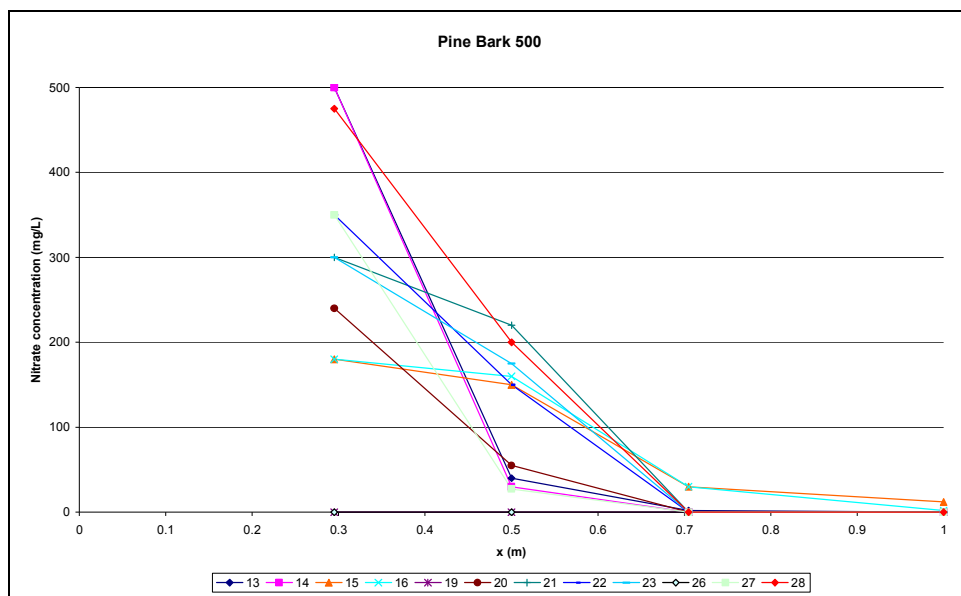


Figure 4.62: Experiment 1 - Evolution of the nitrate concentration over the column length for PB for $C_0 = 500 \text{ mg/l}$ at flow rate 1

The COD of the output for the Pine bark substrate at 500 mg/l are shown in Figures 4.63 and 4.64.

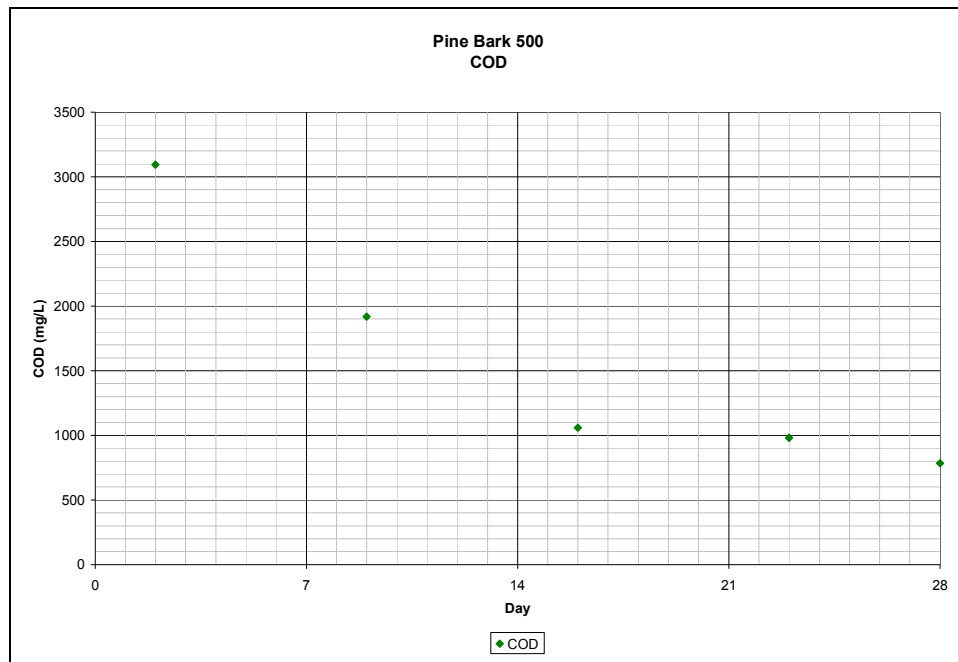


Figure 4.63: Experiment 1 – Evolution of COD for PB for $C_o = 500$ mg/l at flow rate 1

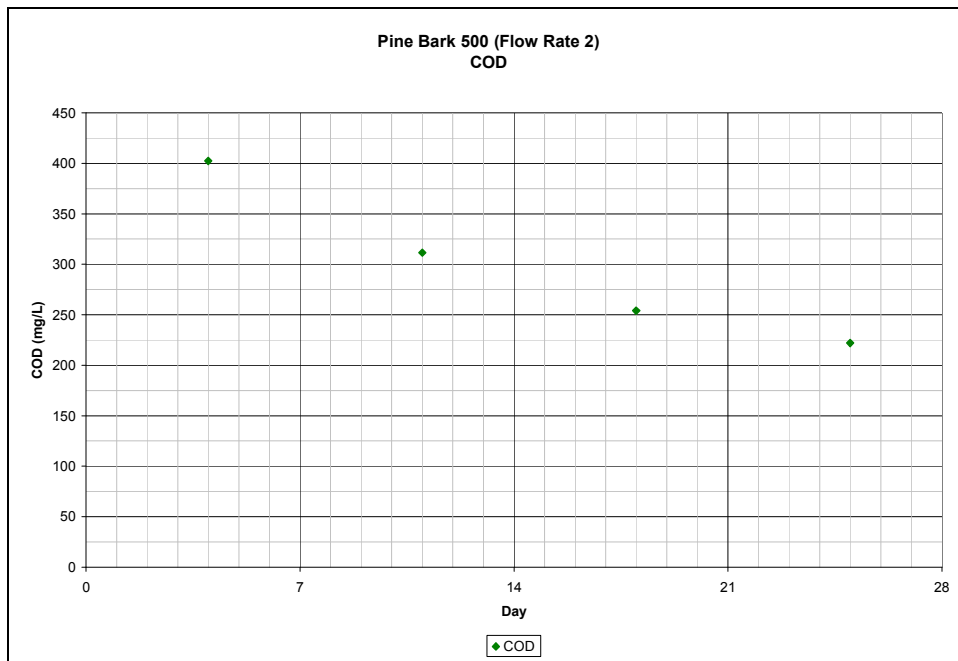


Figure 4.64: Experiment 2 – Evolution of COD for PB for $C_o = 500$ mg/l at flow rate 2

In the column studies, for flow rate 1, the PB showed a better performance than in the batch tests, by completely removing the nitrates after 5 to 7 days. However, during experiment 2, the system failed to reach regime. None the less, a longer testing period and more in depth microbiological analyses are required to draw significant conclusions.

The COD of the output effluent dropped by 75% over the period of experiment 1, from 3100 mg/l to 800 mg/l. In experiment 2, the COD values decreased during the duration of the experiment to a final output of 225 mg/l.

The pH during both experiments rose at a fairly constant rate from an acid nature, until it reached the optimum range for nitrate removal. This buffering capacity is comparable to the drop in nitrate concentration represented in experiment 1. Environmental conditions remained fairly constant throughout both experiments. The temperature ranged between 18 and 22 °C, whereas the $\text{NH}_3 - \text{N}$ reducing to less than 1 mg/l.

$C_o = 2000 \text{ mg/l}$

The evolution of the nitrate concentrations and pH for the Pine bark substrate are shown in Figures 4.65 and 4.66.

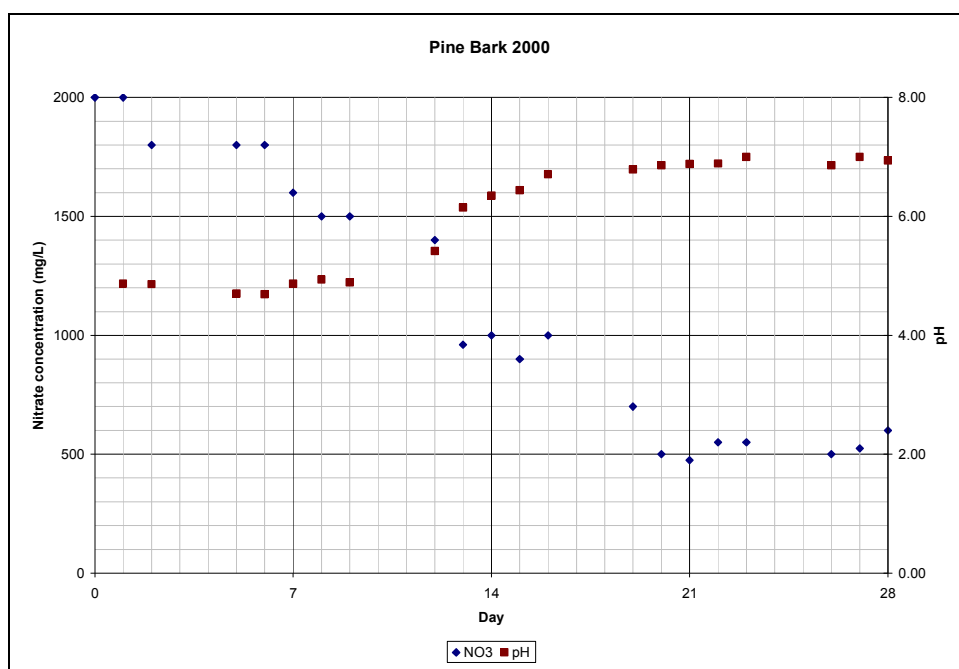


Figure 4.65: Experiment 1 - Evolution of the nitrate concentration and pH for PB for $C_o = 2000 \text{ mg/l}$ at flow rate 1

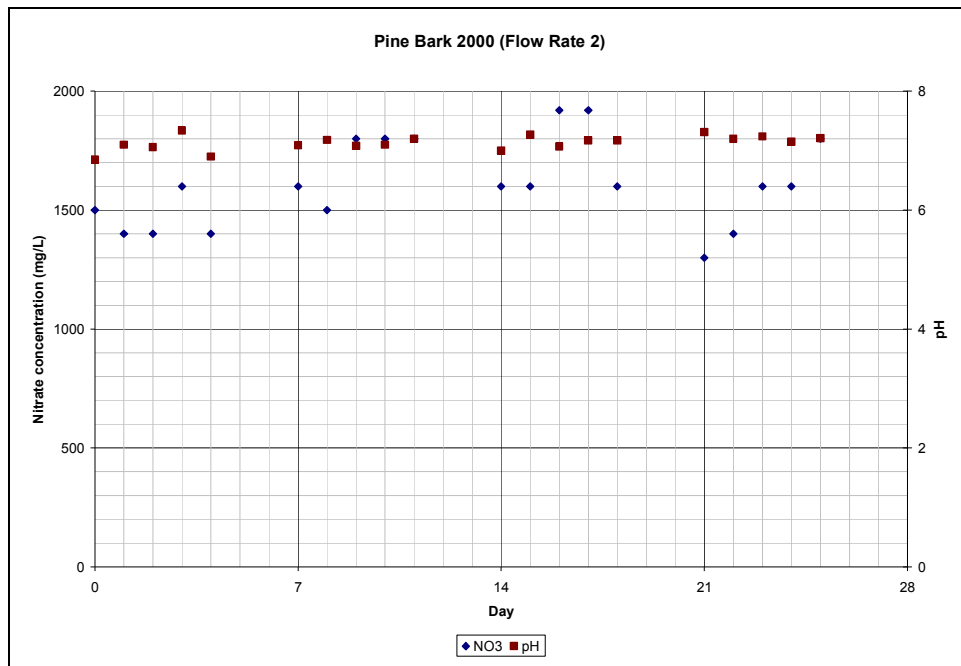


Figure 4.66: Experiment 2 - Evolution of the nitrate concentration and pH for PB for $C_0 = 2000 \text{ mg/l}$ at flow rate 2

The COD of the output for the pine bark substrate at 2000 mg/l are shown in Figures 4.67 and 4.68.

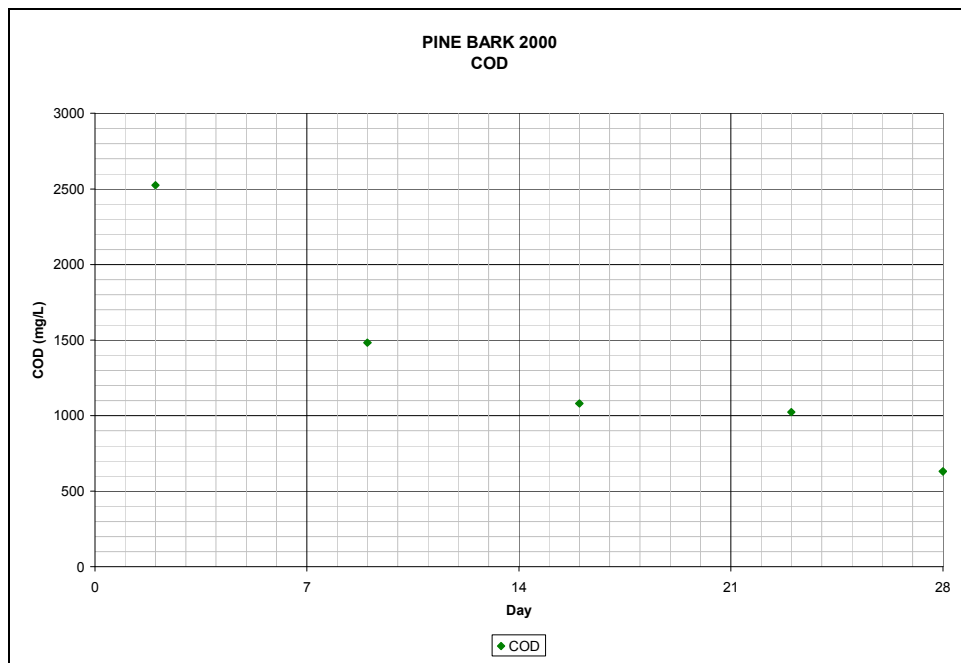


Figure 4.67: Experiment 1 – Evolution of COD for PB for $C_0 = 2000 \text{ mg/l}$ at flow rate 1

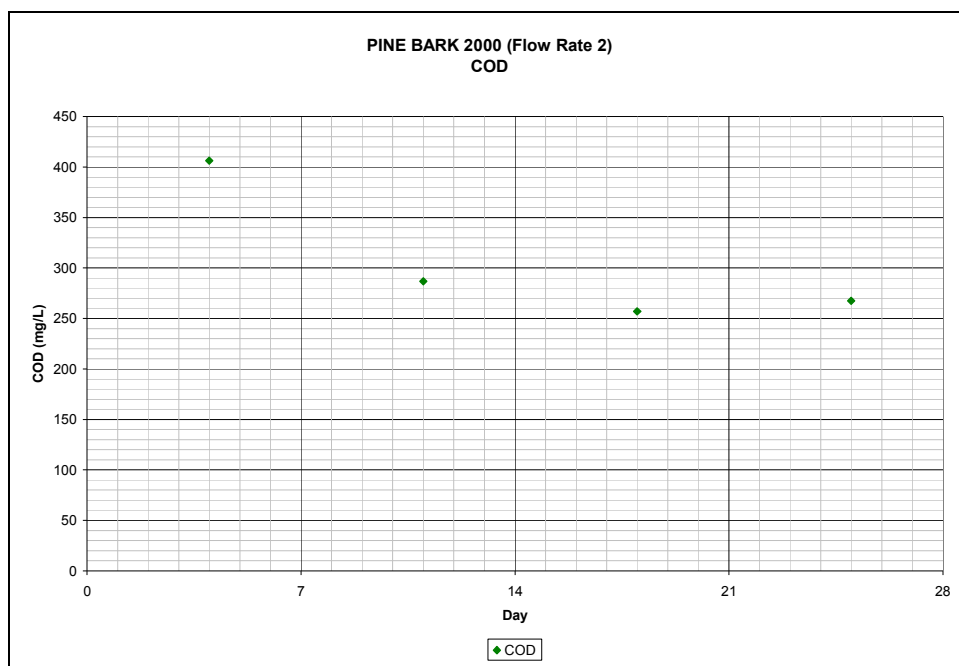


Figure 4.68: Experiment 2 – Evolution of COD for PB for $C_o = 2000$ mg/l at flow rate 2

During the first 6 days of experiment 1 the column showed little change in concentration. This plateau is typical for pine bark due to the low pH value, which inhibits microbial activity. After this point, a more noticeable rate of denitrification was observed. It was particularly evident that during the third week there was a substantial drop in nitrate concentration. This is related to the change in pH, which rose to the optimum range for denitrification, allowing the system to reach 75% efficiency of nitrate. As full denitrification was not achieved it is apparent that the pine bark is releasing carbon at a slower rate than that at which nitrate is being supplemented. It is therefore evident that the contact time was too low and that the substrate requires over 7 days for a zero nitrate level to be reached.

In experiment 2, the nitrate level stayed at a concentration of approximately 1500 mg/l for 8 days, where the peaks and drops were more likely due to errors associated with the nitrate stick method. After day 8 the concentration rose and remained at this level for the remaining 3 days of the week. The lower rate of denitrification achieved can be attributed to the flow rate being too high, resulting in insufficient contact time between the solution and substrate, thus only 35% removal efficiency was achieved against 75% in the first experiment for pine bark at $C_o = 2000$ mg/l.

In experiment 1, the COD of the output effluent dropped by 76% from 2500 mg/l to 600 mg/l, whereas, the COD values during experiment 2 decreased to 260 mg/l over the first three weeks of testing and remained at this level until the end of the experiment.

Initially the pH during the experiment 1 stayed at a constant level of 4 – 5. After 9 days, however, the pH tended to increase to neutrality, whilst pH during experiment 2 stayed constant at approximately 7. The temperature remained in a range between 19 and 22 °C. The $\text{NH}_3 - \text{N}$ during experiment 1 did increase after the first week of testing, however decreases to remain below 3 mg/l until the completion of the experiment, whilst the recorded $\text{NH}_3 - \text{N}$ of experiment 2 was less than 1mg/l throughout the duration of the experiment.

4.4.4.3. Immature CGR compost (CGR 10)

$$C_o = 500 \text{ mg/}\ell$$

The evolution of the nitrate concentrations and pH for the CGR 10 substrate are shown in Figures 4.69 and 4.70.

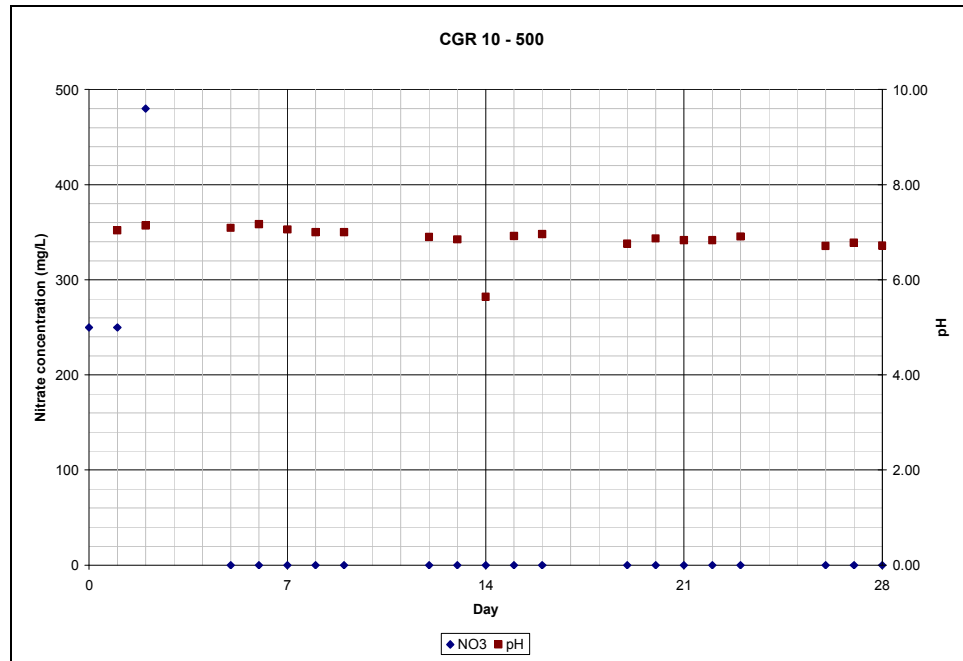


Figure 4.69: Experiment 1 - Evolution of the nitrate concentration and pH for CGR 10 for $C_o = 500 \text{ mg/}\ell$ at flow rate 1

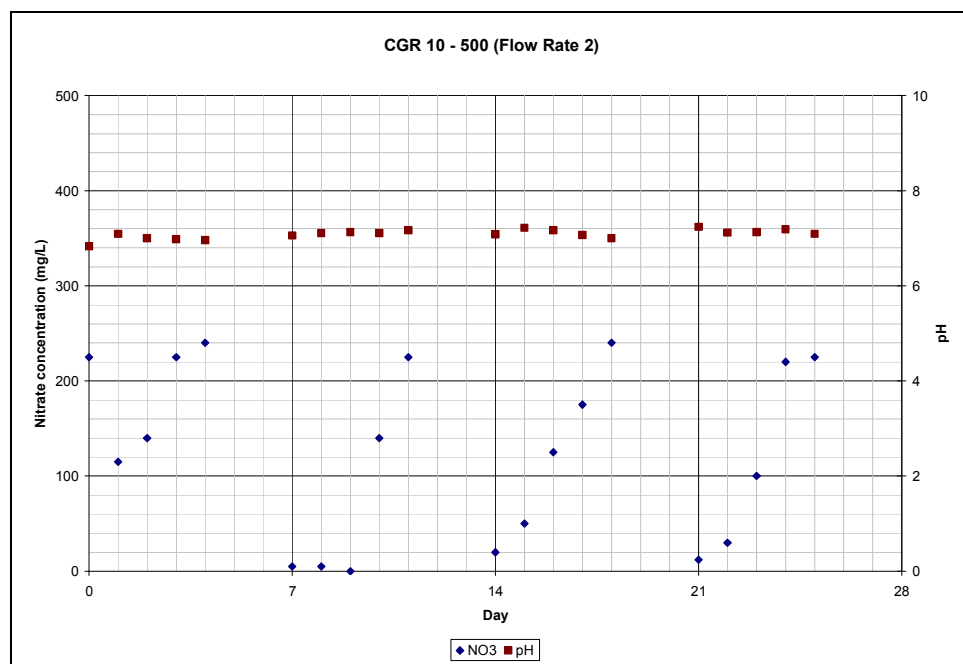


Figure 4.70: Experiment 2 - Evolution of the nitrate concentration and pH for CGR 10 for $C_o = 500 \text{ mg/}\ell$ at flow rate 2

The evolution of the nitrate concentration over the length of the column for flow rate 1 is shown in Figure 4.71.

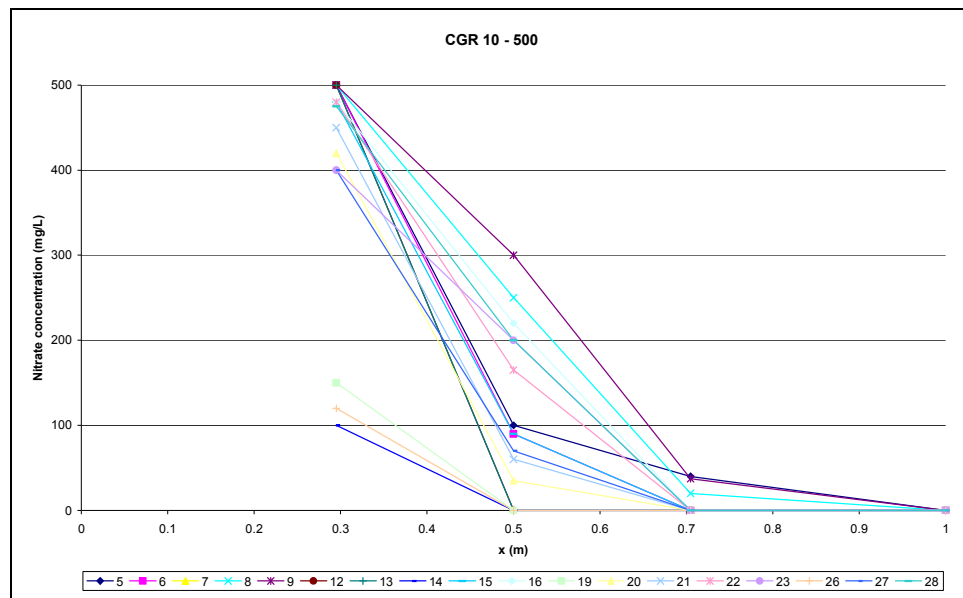


Figure 4.71: Experiment 1 - Evolution of the nitrate concentration over the column length for CGR 10 for $C_0 = 500 \text{ mg/l}$ at flow rate 1

The COD of the output for the CGR 10 substrate at 500 mg/l are shown in Figures 4.72 and 4.73.

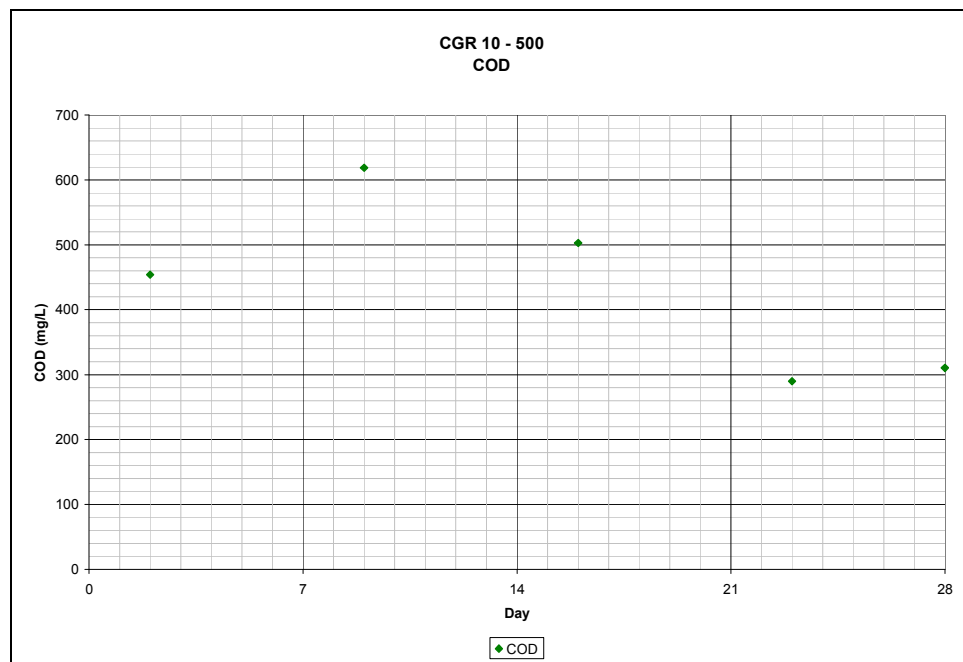


Figure 4.72: Experiment 1–Evolution of COD for CGR10 for $C_0 = 500 \text{ mg/l}$ at flow rate 1

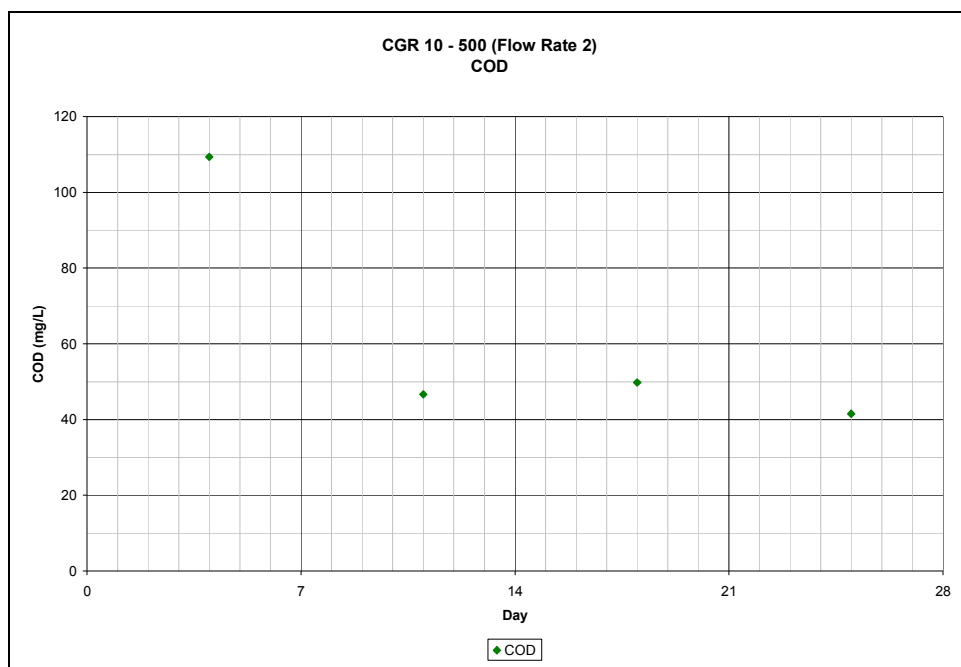


Figure 4.73: Experiment 2–Evolution of COD for CGR10 for $C_o = 500 \text{ mg/l}$ at flow rate 2

Figure 4.70 indicates that in experiment 1, nitrates were being removed within 5 – 7 days. In experiment 2, the column failed to reach full denitrification achieving 96% removal, which leads us to conclude that the substrate in the column required more than 4 days for total nitrate removal to occur.

The COD figures showed an increase from week 1 to 2. This could be as a result of readily biodegradable carbon being released. After week 2, the COD values dropped steadily by 50% to approximately 300 mg/l . During experiment 2, the COD dropped from week 1 – 2 by 55% to below 50 mg/l and stayed at this level until the conclusion of the experiment.

pH levels throughout the tests stayed constant at approximately 7, whilst the temperature ranged between 19 and 22°C . The $\text{NH}_3 - \text{N}$ showed a slight increase over the first two weeks, before reducing to less than 1 mg/l .

$C_o = 2000 \text{ mg/l}$

The evolution of the nitrate concentrations and pH for the CGR 10 substrate are shown in Figures 4.74 and 4.75.

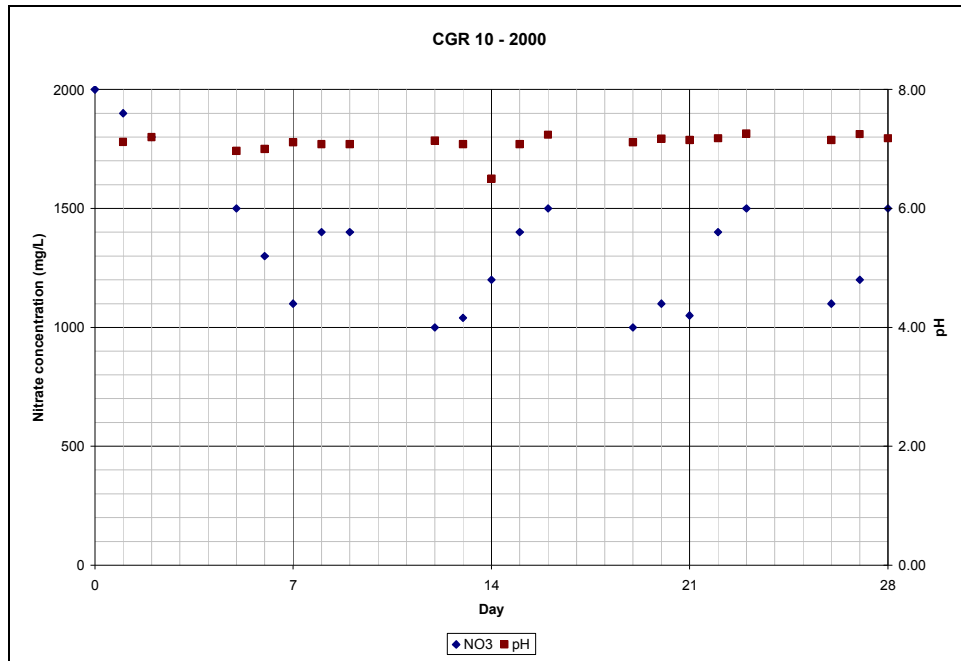


Figure 4.74: Experiment 1 - Evolution of the nitrate concentration and pH for CGR 10 for $C_o = 2000 \text{ mg/l}$ at flow rate 1

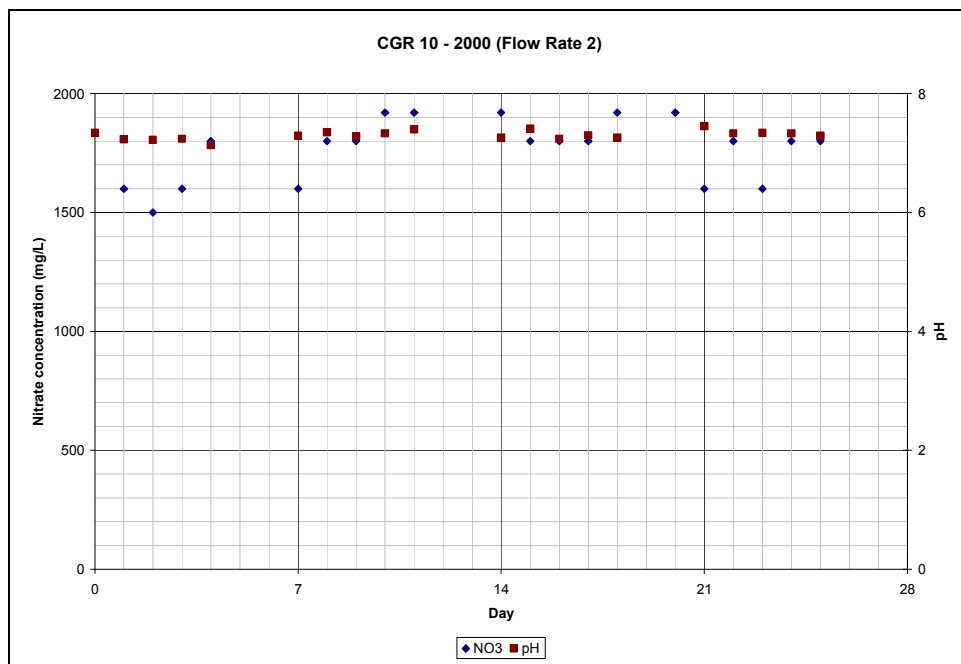


Figure 4.75: Experiment 2 - Evolution of the nitrate concentration and pH for CGR 10 for $C_o = 2000 \text{ mg/l}$ at flow rate 2

The COD of the output for the CGR 10 substrate at 2000 mg/l are shown in Figures 4.76 and 4.77.

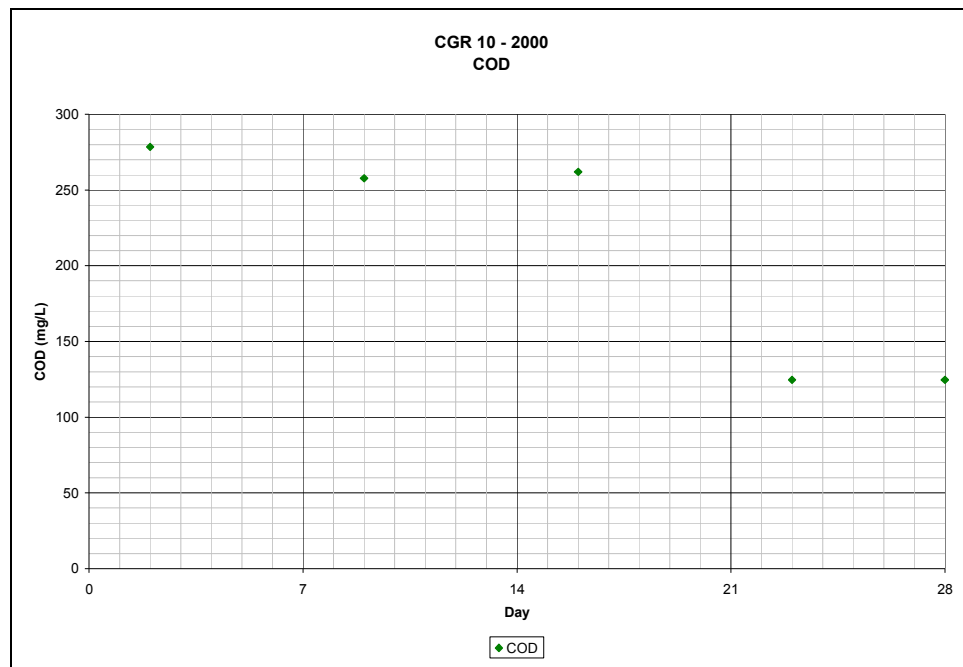


Figure 4.76: Experiment 1 – Evolution of COD for CGR 10 for $C_o = 2000$ mg/l at flow rate 1

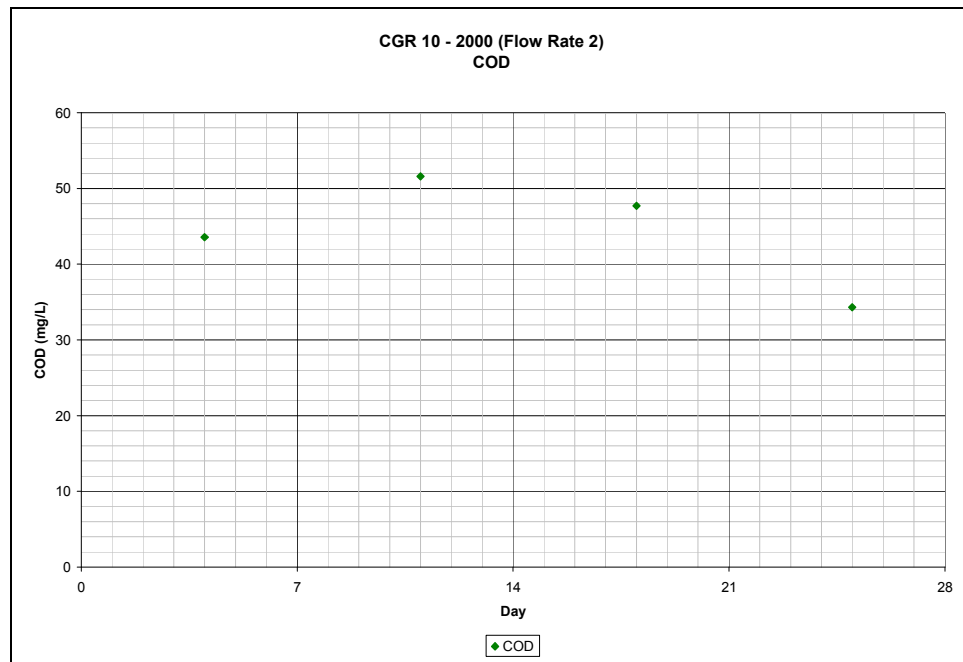


Figure 4.77: Experiment 2 – Evolution of COD for CGR 10 for $C_o = 2000$ mg/l at flow rate 2

During the first week of experiment 1, the nitrate concentration reduced steadily at a linear rate of 130 mg/l per day. After 7 days the nitrate concentration increased by a value of 300 mg/l until the end of the week. The column never achieved full denitrification and only reached a 50% removal of nitrates.

For experiment 2, the nitrate level stayed at a concentration of 1600 mg/l for initial 4 days. After 7 days the concentration rose to 1800 mg/l and remained at this level for the remainder of the experiment. The column failed to achieve full denitrification during the 4 week period. The CGR 10 substrate showed minimal denitrification which can be contributed to the flow rate being too high, resulting in insufficient contact time, thus only a maximum of 25% removal efficiency was achieved as appose to 50% removal in the first experiment for CGR 10 at $C_o = 2000$ mg/l. As full denitrification was not achieved, it is apparent that the CGR 10 was releasing carbon at a slower rate than that at which nitrate was being supplied.

The evolution of COD suggests that the flow rate was too high to allow for a significant bio-leaching of carbon, as experienced in most of the experiments at low rate 2.

The pH measured during the period of the tests stayed at a constant level between 7 and 7.25. The temperature remained constant for both experiments, in the range between 19 and 22 °C, whilst the measured $\text{NH}_3 - \text{N}$ during experiment 2 decreased from 4.5 mg/l after the first week to less than 1 mg/l at the end of the experiment. In experiment 1, the $\text{NH}_3 - \text{N}$ decreased from 6 mg/l after the first week to between 1.5 and 3 mg/l and remained at that level for the remainder of the experiment.

In summary, the poor performance of all substrates at flow rate 2, for both concentrations, suggest that the short contact time was not long enough to establish an active bio-film for denitrification.

This argument cannot be verified as part of this research and will be subject of future studies.

4.4.5. Loading Rates and Hydraulic Retention Time

The Hydraulic retention time (HRT) is a measure of the average length of time that a soluble compound remains in a constructed bioreactor and is calculated by the volume of the reactor divided by the flow rate (<http://www.lenntech.com/wwtp/hrt.htm> accessed 19/12/2009).

The hydraulic retention time has an affect on nitrate removal and is thus vitally important in the design of a bioreactor for nitrate removal (Tsui et al., 2007). The hydraulic loading rate is a critical factor for the design of treatment systems and is determined as the volume per day that can be applied over a surface area (Zhou et al., 2007).

Table 4.18 presents the performance of the various substrates for each of the columns for the changes in concentration and flow rate. These results can be extrapolated using simple ratio concentrations to provide an estimate of the ideal flow rates and hydraulic retention times.

Table 4:18: Summary of the performance of the column studies over both experiments

Substrate	NO ₃ Conc. (mg/l)	Flow Rates (l/day)		HRT (Days)		% Removal		Loading Rate (l/m ² /day)	
		Exp. 1	Exp. 2	Exp. 1	Exp. 2	Exp. 1	Exp. 2	Exp.1	Exp.2
CGR RAW	500	2.48	5.625	8.06	3.56	100	100	123.32	279.71
PB	500	2.00	5.00	10.00	4.00	100	90	99.45	248.63
CGR 10	500	1.7	2.85	11.76	7.02	100	96	84.54	141.72
CGR RAW	2000	2.38	5.65	8.40	3.54	100	45	118.35	280.95
PB	2000	2.00	5.00	10.00	4.00	75	35	99.45	248.63
CGR 10	2000	1.78	2.85	11.24	7.02	50	25	88.51	141.72

For both the tests conducted at $C_o = 500$ mg/l and 2000 mg/l, the CGR RAW was the best performing substrate. For the test at $C_o = 500$ mg/l full nitrate removal was achieved at both flow rates.

Due to the 100% nitrate removal achieved at $C_o = 500$ mg/l at both flow rates it can be concluded that the CGR RAW can sustain a higher flow rate than 5.625 l/day as well as a loading rate above 280 l/m²/day. The HRT time required for full nitrate removal is less than 3.5 days.

For the tests conducted at $C_o = 2000 \text{ mg/l}$, the system only achieved full nitrate removal at the first flow rate of 2.38 l/day in experiment 1, whereas in experiment 2 a 45% nitrate removal was reached. Through simply extrapolation an estimated flow rate of 2.54 l/day and a HRT of 8 days would be needed for the system to achieve full denitrification.

The pine bark was the least efficient substrate at $C_o = 500 \text{ mg/l}$ achieving 100% nitrate removal at the flow rate in experiment 1, however only reaching 90% nitrate removal in experiment 2. This suggests that the flow rate required for full denitrification is between $2 - 5 \text{ l/day}$. A flow rate of 4.5 l/day and a HRT of 4.5 days are estimated.

At $C_o = 2000 \text{ mg/l}$, the pine bark only achieved 75% nitrate removal in experiment 1 and 35% in experiment 2. This indicates that both flow rates were too high for full denitrification to be reached. A flow rate of $1.5 - 1.75 \text{ l/day}$ and a HRT of 13 days are estimated.

The CGR 10 at $C_o = 500 \text{ mg/l}$ also achieved 100% nitrate removal at the flow rate in experiment 1, however only reached 96% nitrate removal in experiment 2. This suggests that the flow rate required for full denitrification is between $1.7 - 2.85 \text{ l/day}$. A flow rate of 2.74 l/day and a HRT of 7.3 days are estimated.

At $C_o = 2000 \text{ mg/l}$, the CGR 10 was the least efficient substrate only obtaining 50% nitrate removal in experiment 1 and 25% in experiment 2. This indicates that both flow rates were too high for full denitrification to be reached. A flow rate of $0.7 - 0.9 \text{ l/day}$ and a HRT of 22 - 28 days are estimated.

4.5. Durability Testing

Durability testing was done on 4 columns that had been used in previous years of study to ascertain whether the substrates still had denitrification capabilities over an extended period of time.

The columns were called Pine Bark and Immature Compost Björn (PB – B and IC – B) and Pine Bark and Immature Compost Giulia (PB – G and IC – G).

Due to the extent of the testing duration the graphs were not included. They, along with the raw data, can be seen in Appendix D.

Prior to beginning the durability test, it was noticed that the IC – G column was highly compacted and contained a large portion of fine materials. The moisture content within the column was also particularly high.

Once the testing procedure began, the nitrate concentration of the initial output effluent, in the IC – G column after zero days was less than 500 mg/l which can be attributed to dilution of the nitrate solution. However, the column did show denitrification and obtained a full denitrification on numerous occasions during the testing period.

The testing procedure became hampered by solids blocking the bottom filter and considerably reducing the output flow. It was also noticed that the nitrate solution was channelling through the column, causing degradation, as apposed to percolating through the substrate material. After 3.5 months of testing, this particular column stopped showing any denitrification and was thus discontinued after 4 months.

For the PB – G column full denitrification was achieved within 10 days for the first three weeks. However after this period, the time needed for the column to reach full denitrification became longer after each refilling, thus the rate of denitrification had decreased. After a period of 5 months the substrate failed to achieve any further denitrification with only 50% nitrate removal.

The two 'younger' columns IC – B and PB – B displayed similar characteristics during the experimental period. During the first 3 months both were able to achieve full denitrification within 4 – 5 days. After which the rate of removal decreased roughly on a monthly basis, taking 8 – 10 days for full denitrification to occur from the 5th month.

Finally, after 6 months, both columns failed to denitrify the nitrate solution any further and stayed at a constant nitrate concentration level of 80% nitrate removal.

An accumulation of the COD output for each of the columns over the 7 month testing period is shown in Figure 4.78.

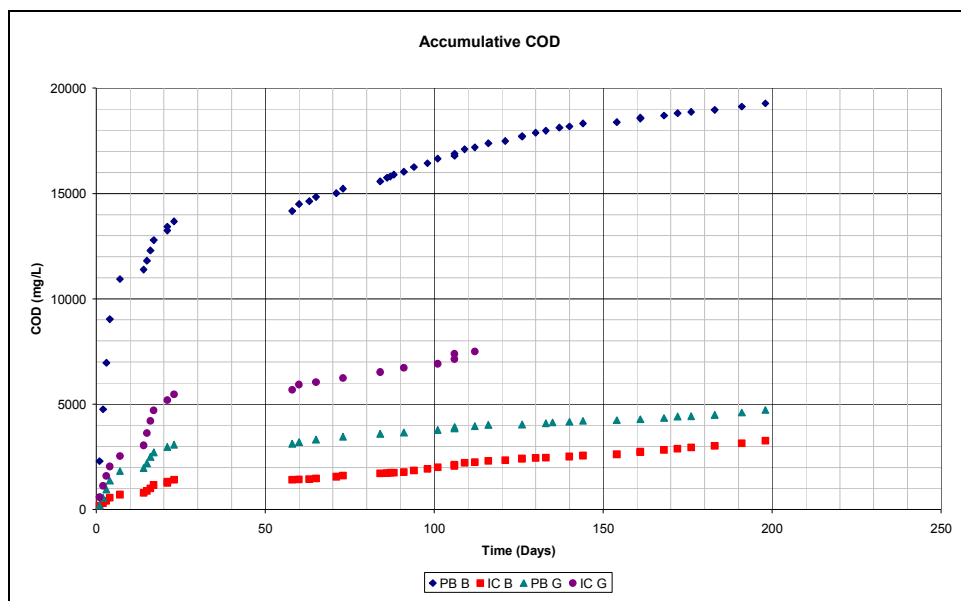


Figure 4.78: Durability Test - COD Accumulation

It is evident from Figure 4.78 that the 'younger' column of pine bark had a considerably greater output of COD over the testing period than the other three substrates. This observation can be attributed to the slowly biodegradable carbon present in the pine bark. This carbon had now broken down and become available for denitrification. The carbon released is in the form of COD. As the testing period increased the output of COD reduced, causing the graphs to flatten out. This indicates that less carbon was being released and correlates to the decrease in rates of denitrification observed.

It was clear from the results obtained that, in both cases (Columns B and G), the pine bark substrate was more effective at achieving full denitrification than the two composted materials and was able to do so after a longer period of use in the columns.

This can be attributed to the fact that, firstly there is no longer a retarding effect for denitrification due to the inhibiting nature of the pine bark's acidic pH. The system had reached acclimatisation and its regime through alkalinity provided by the OH^- ions produced during denitrification thus buffering the pH into the optimum range for

bacterial growth and thus denitrification. Secondly the large molecules of slowly biodegradable carbon have now been broken down and are available for the denitrifying micro-organisms.

4.6. Biogas Analysis

A gas analysis was also conducted for each of the columns on days when adequate levels of biogas were available.

During all the column experiments, nitrogen gas was pumped into the columns during drainage to prevent a vacuum effect as well as to keep the columns in an anaerobic condition. Thus, during drainage and refilling the biogas measurement equipment was isolated. However due to excess nitrogen gas flowing into the columns, once the gas system was re-opened, the nitrogen gas would alter the quantity of gas produced. It was thus decided to only measure the percentage of methane (CH_4), carbon dioxide (CO_2) and oxygen (O_2) produced to ensure that the columns did not become methanogenic, which would be noticed in terms of excessive CH_4 production and decline of denitrification.

Only 4 of the 6 columns produced a sufficient quantity of gas to make gas analysis possible.

Both the PB and CGR 10 columns at 500 mg/l produced similar ranges for each of the gases.

Methane (CH_4)	0.1%
Carbon dioxide (CO_2)	0.2 – 0.6%
Oxygen (O_2)	3.9 – 4.8%

The PB and CGR RAW columns at 2000 mg/l showed slightly different results for each of the gases. The Methane (CH_4) ranged between 0.1 and 0.2 % in both the columns throughout all the experiments. The Carbon dioxide (CO_2) level in the PB column started off at 11.4 % before dropping to below 1.0%. This initial high value is due to the release of carbon in the form of CO_2 . The percentage Oxygen (O_2) however rose from 1.1% to above 4.1%.

In the CGR RAW column the Carbon dioxide (CO_2) output stayed between 0.6 and 1.0% whereas the percentage Oxygen (O_2) increased from 2.1 – 6.4%.

As the levels of methane in the columns stayed relatively low, it is safe to assume that they did not become methanogenic. The increase in oxygen may be due to leaks within the construction of the columns or through infiltration when adding the nitrate solution.

4.7. Summary of Results

Tables 4.19 and 4.20 are a summary of the performance of each of the substrates during the batch and column tests.

Table 4.19: Summary of batch test results at each nitrate concentration

Substrate	Input			% Removal	Rate of Removal (Days ⁻¹)	COD (mg/ℓ)	NH ₃ (mg/ℓ)
	C _o	C/N	pH				
Pine Bark	100	62.15	4.18	100	46.775	5021	2.25
CGR RAW	100	90.19	5.45	100	588	4325 – 5212	18.5
DGR 10	100	12.75	7.40	100	79.74	7822	8.5
CGR 10	100	23.91	6.98	100	94.43	2754	2.5
DAT	100	22.96	6.93	100	97.46	4165	4.3
TW	100	17.60	7.27	100	130.31	4629	2.3

Substrate	Input			% Removal	Rate of Removal (Days ⁻¹)	COD (mg/ℓ)	NH ₃ (mg/ℓ)
	C _o	C/N	pH				
Pine Bark	500	62.15	4.18	55	38.183	14157	22.5
CGR RAW	500	90.19	5.45	100	1408	3951 - 7200	25
DGR 10	500	12.75	7.40	100	113.66	17783	87.2
CGR 10	500	23.91	6.98	100	80.35	3177	3.0
DAT	500	22.96	6.93	100	66.89	7442	28.0
TW	500	17.60	7.27	100	133.86	7396	12.0

Substrate	Input			% Removal	Rate of Removal (Days ⁻¹)	COD (mg/ℓ)	NH ₃ (mg/ℓ)
	C _o	C/N	pH				
Pine Bark	2000	62.15	4.18	20	126.250	13245	30
CGR RAW	2000	90.19	5.45	100	181	7009 - 7870	85.75
DGR 10	2000	12.75	7.40	100	61.74	-	-
CGR 10	2000	23.91	6.98	100	164.26	-	-
DAT	2000	22.96	6.93	100	41.27 - 48.60	13712	14.3
TW	2000	17.60	7.27	100	71.71 - 117.94	7398 - 12359	8.6

Fresh Substrates
Immature Substrates
Mature Substrates

Table 4.20: Summary of the column tests at the 2 different flow rates

Substrate	Input			Flow Rates (ℓ/day)		HRT (Days)		% Removal		Loading Rate (ℓ/m ² /day)	
	C _o	C/N	pH	Exp. 1	Exp. 2	Exp. 1	Exp. 2	Exp. 1	Exp. 2	Exp. 1	Exp. 2
CGR RAW	500	62.15	4.18	2.48	5.625	8.06	3.56	100	100	123.32	279.71
Pine Bark	500	90.19	5.45	2.00	5.00	10.00	4.00	100	90	99.45	248.63
CGR 10	500	23.91	6.98	1.7	2.85	11.76	7.02	100	96	84.54	141.72
CGR RAW	2000	62.15	4.18	2.38	5.65	8.40	3.54	100	45	118.35	280.95
Pine Bark	2000	90.19	5.45	2.00	5.00	10.00	4.00	75	35	99.45	248.63
CGR 10	2000	23.91	6.98	1.78	2.85	11.24	7.02	50	25	88.51	141.72

Substrate	Input			COD (mg/ℓ)		NH ₃ (mg/ℓ)	
	C _o	C/N	pH	Exp. 1	Exp. 2	Exp. 1	Exp. 2
CGR RAW	500	62.15	4.18	10255	624	49.0	11.0
Pine Bark	500	90.19	5.45	7841	1190	21.0	27.0
CGR 10	500	23.91	6.98	2176	247	7.0	23.0
CGR RAW	2000	62.15	4.18	7397	434	39.0	16.0
Pine Bark	2000	90.19	5.45	6742	1218	12.5	0.0
CGR 10	2000	23.91	6.98	1048	177	15.0	6.5

CHAPTER 5

5. CONCLUSIONS AND RECOMMENDATIONS

The results of the laboratory experiments found in Chapter 4 substantiate that all six of the substrates prove to be effective as carbon sources to denitrify various concentration levels of nitrified leachate, at different degrees of efficiency.

The six substrate materials had varying compositions of relatively high carbon (C) content in comparison to nitrogen (N). This characteristic makes these materials well suited for nitrate removal as they provide organic carbon for denitrification without increasing the nitrogen concentration. They also act as a medium for denitrifying bacteria.

The characterisation tests indicated that the fresh materials had higher carbon to nitrogen ratios than the composted substrates. The CGR RAW substrate had the highest carbon to nitrogen ratio of 90.19 and although the pH value of 5.45 falls just outside the optimum range for denitrification of 6 – 8, it was expected that this would be the best performing substrate. The pine bark substrate had the second highest carbon to nitrogen ratio of 62.15 however due to the acidic nature of the material, with a pH of 4.18; which would be inhibitory to denitrification it was likely that this substrate would not perform as well.

As seen in Table 4.19, all the batch tests showed positive results, with regard to achieving full denitrification with a 100% removal occurring in 5 of the 6 substrates, at all the different nitrate concentrations. The only substrate not to achieve full denitrification was the pine bark. It only managed to achieve 100% removal at a nitrate concentration of 100 mg/l. During the tests conducted at 500 and 2000 mg/l, only 55% and 20% removal were achieved. The best performing substrate was the CGR RAW which achieved full denitrification at the highest nitrate concentration of 2000 mg/l between 9 – 12 days, which can be attributed to its high C/N ratio.

All the small scale batch tests demonstrated similar characteristics of an acclimatisation period before decreasing linearly with time. The duration of the acclimatisation period was strongly related to that of the initial input concentrations of the nitrate solution.

Using the results acquired in both the characterisation and batch tests, three substrates were chosen to be used in column tests.

The column tests reflected promising results at $C_o = 500 \text{ mg/l}$ during experiment 1, with all 3 achieving full denitrification. At $C_o = 2000 \text{ mg/l}$ only the CGR RAW column reached full denitrification. The pine bark and the CGR 10 substrates only managed 75% and 50% removal respectively. Once again the CGR RAW substrate columns reflected the best results. The column at 500 mg/l displayed a HRT of 8.06 days was required whereas the higher concentration of 2000 mg/l required a HRT of 8.40 days.

During experiment 2, however the increased flow rates were too high to allow denitrifying bacteria sufficient contact period or hydraulic retention time to establish themselves. The CGR RAW substrate column at 500 mg/l was the only one to achieve 100% nitrate removal. A HRT time required for full denitrification is less than 3.54 days.

It is noted that flow through the columns improves the organic matter release and dispersion rates compared to a system where the effluent remains stagnant (Diaz et al., 2003). However a flow rate that is too high could result in an insufficient hydraulic retention time, which does not allow denitrifying bacteria to accumulate for denitrification. The results also indicate that the rate at which carbon is being released is slower than the rate at which nitrates are being added.

The main concern of this treatment method is the increase in COD concentration produced by organic matter release. The COD levels were all above the limits provided by DWAF (DWAF - General Authorisations in terms of Section 39 of the National Water Act, 1998). It was found that over time the COD concentrations did decrease, but, in most cases, not sufficiently to fall into DWAF's Water Quality criteria (DWAF - General Authorisations in terms of Section 39 of the National Water Act, 1998).

The eThekweni landfills receive large volumes of garden refuse monthly which is separated from the main waste stream. Large quantities of pine bark are produced by both SAPPI and Mondi paper as a by product of the paper and pulp industry in South Africa. If needed for the denitrification process the pine would be obtainable for utilisation. These two materials are highly abundant and easily available on site, thus making them fairly inexpensive.

They could therefore be successfully employed at local landfill sites to denitrify treated leachate which would prevent excessive treatment costs as well as support the development of a real waste management strategy that is in the process of being implemented within the country. The reactor would not be either labour or energy intensive due to its natural open circuit system making it a low energy treatment solution.

Further studies need to be done at different flow rates and concentrations to ensure that the reactor is robust and flexible to deal with the change in quality of the leachates during the life of the landfill. Lower concentrations need to be investigated to determine whether the substrates are suitable for all ranges of nitrates and leachates.

In this research the use of a synthetic nitrate solution was used to simulate the treated leachate from a landfill, so as to operate the process in controlled conditions and eliminate the disturbances in the NO_3 analysis due to the presence of chlorinated compounds in the leachate as experienced in previous research studies (Pisano, 2007). However tests with the treated leachate would be recommended, in order to ascertain a more accurate understanding of how the substrates might behave in a real full-scale treatment system.

The use of a combination of substrates as well as different levels of maturity is also required to determine the ideal material for implementation in a full-scale reactor in the future. Larger scale reactors and different reactor configurations need to be investigated.

Investigations into the effects of increasing the pH of the synthetic nitrate solution to balance or buffer the acidic nature of the pine bark by using a light alkali such as Sodium Carbonate need to be conducted.

Analyses of the C/N ratios of the output material were conducted on the wet material. This did not provide an accurate balance of the released organics, as carbon leached out from the substrate may be still trapped in the biofilm developed inside of the pores. It would thus be recommended that C/N ratios be calculated on the dry matter so as to try and accurately calculate and predict a mass balance.

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Appendix A: Characterisation Tests

Appendix B: Batch Tests

Appendix C: Column Tests

Appendix D: Durability Tests

Appendix E:

Manuscript Number: HAZMAT-D-10-00376

Title: Effect of pine bark and compost on the biological denitrification process of non-hazardous landfill leachate: focus on the microbiology

Authors: Cristina Trois, BScEng and MScEng, PhD; Frédéric Coulon, PhD; Cécile Polge de Combret, BScEng and MSc; Jean M.F. Martins, PhD; Laurent Oxarango, PhD

Manuscript Number: HAZMAT-D-10-00376

Title: Effect of pine bark and compost on the biological denitrification process of non-hazardous landfill leachate: focus on the microbiology

Article Type: Short Communication

Keywords: Keywords: denitrification; landfill leachate; compost; pine bark; microbial diversity

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Order of Authors: Cristina Trois, BScEng and MScEng, PhD; Frédéric Coulon, PhD; Cécile Polge de Combret, BScEng and MSc; Jean M.F. Martins, PhD; Laurent Oxarango, PhD

Abstract: In an attempt to optimize the cost-efficiency of biological denitrification for high strength landfill leachate, our study focused on finding low-cost alternatives to traditional expensive chemicals. The overall objective was to assess the behaviour of the bacterial community in relation to organic substrates such as garden refuse compost and pine bark. Denitrification processes in fixed bed reactors were simulated at laboratory scale using anaerobic batch tests, with a synthetic solution at a nitrate concentration of 500 mg l⁻¹. Results suggest that pine bark released large amounts of phenolic compounds and hydroxylated benzene rings, which both can delay the acclimatization time and inhibit the biological denitrification (only 30% efficiency). The presence of potential pathogens like *Enterobacter* and *Pantoea agglomerans* prevents its applicability in full-scale operations. Whereas, lightly composted garden refuse (CGR) offered an adequate substrate for the formation of a biofilm necessary to achieve full denitrification within 7 days. CGR contributed to a rapid establishment of an active consortium of denitrifiers including *Acinetobacter*, *Rhizobium*, *Thermomonas Rheinheimera*, *Phaeospirillum* and *Flavobacterium*. Clearly, composition, nature, carbon to nitrogen ratio (C/N) and degree of maturity of the substrates impact directly on the development of the bacterial population and, therefore, on the long-term removal efficiency.

Dear Journal of Hazardous Materials,
Please find attached the manuscript:

Effect of pine bark and compost on the biological denitrification process of non-hazardous landfill leachate: focus on the microbiology

Cristina Trois^{a*}, Frédéric Coulon^b, Cécile Polge de Combret^b, Jean M.F. Martins^c, Laurent Oxarango^c,

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Hope everything is in order.

Kind regards

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Journal of Hazardous Materials

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Response 1: The abstract has been revised and it is now exactly 200 words.

Comment 2: The references should be cited sequentially in the text.

Response 2: Done

Comment 3: Reference 5 provided in the manuscript is not cited in the text.

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Effect of pine bark and compost on the biological denitrification process of non-hazardous landfill leachate: focus on the microbiology

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Keywords: denitrification; landfill leachate; compost; pine bark; microbial diversity

1. Introduction

The majority of municipal solid waste landfills, including those that previously co-disposed hazardous materials continue to receive a significant proportion of bioreactive wastes which produce mainly greenhouse gases and wastewater known as leachate [1]. Landfill leachate contains organic and inorganic pollutants including humic acids, ammonia, heavy metals, persistent organic pollutants and inorganic salts at high concentrations (e.g. Chemical Oxygen Demand (COD) between 2000 - 6000 mg l⁻¹, ammonia between 1000-1600 mg l⁻¹ and chloride between 1500-2600 mg l⁻¹) [2]. If they are not collected carefully and not discharged safely, they may become a potential pollution source which threatens soil, surface water and groundwater [3]. Therefore, landfill leachate is recognized as an important environmental problem by modern societies. In the treatment of landfill leachate, biological systems such as nitrification-denitrification processes are frequently used [4, 5, 6]. Even though, these systems ensure a high Biological Oxygen Demand (BOD) removal efficiency, they are usually insufficient in degrading high-molecular-weight fractions and decolouring, and their efficiency is often susceptible to the presence of toxic substances and presence of refractory organics such as humic acids and surfactants [7]. In old sanitary landfills, the amount of organic materials having high molecular weight in leachate is high [7]. In the treatment of these wastewaters, therefore, combined systems including many processes such as aerobic–anaerobic decomposition, chemical oxidation, coagulation–flocculation and adsorption are preferred to single-process solutions [1]. However these combined treatment processes are often costly in terms of capital investment, energy requirements and frequent use of additional chemicals [1,7]. Other methods such as reverse osmosis, active carbon adsorption and advanced oxidation processes have been recently

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5 pointed out as more versatile methods, however they only transfer the pollutants without
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7 solving the environmental problem [1,8]. Moreover, their full-scale application is not often
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9 economically feasible.
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13 Clearly there is a need to re-evaluate the methods to remove contaminants from
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15 landfill leachate in order to shift from “waste treatment” to exploitation of landfill leachate
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17 as a resource that can be processed for recovery of energy, nutrients and other constituents.
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22 Biological denitrification is one of the most promising and versatile approaches in the
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24 treatment of landfill leachate [7, 8]. In this process, an external organic substrate (i.e.
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26 methanol, ethanol, acetic acid) or electron donor is needed [9, 10]. While these compounds
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28 are expensive and potentially dangerous, some complex substrates such as tree barks, wood
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30 chips, corncobs, sawdust, compost [11] and newspapers [12] have proved to be efficient
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32 carbon sources for denitrification and generally more suited to treat high strength effluents
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34 [7, 11]. These natural substrates are normally cheaper than the synthetic ones and can be
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36 derived from a typical waste stream [13, 14].
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43 Biological denitrification of landfill leachate is often undertaken in sequencing batch
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45 reactors (SBR) [7] or in constructed wetlands (CW) [15]. Both treatments are known for
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47 their flexibility in terms of adaptation to leachate nature and collection strategies [16, 17].
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49 However, the influence that specific substrates have on the development and nature of
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51 active microbial populations is not yet widely understood [15]. Indeed, performance and
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53 stability of a bio-denitrification process, as of any biological process, depend on the
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55 concentration of the active species and on their metabolic activity. Little is known about
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5 their diversity, distribution, metabolic potential and functional roles. The nitrate-based
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7 microbial communities of which they are members remain uncertain as well as the identity
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9 of their major and minor players and the ecological parameters that influence
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11 denitrification. This information is crucial to better understand the bio-denitrification
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13 process particularly in high strength landfill leachate and for the development of
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15 knowledge-based technologies to accelerate and optimize this treatment.
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20 The objective of this study was to investigate the influence of garden refuse compost
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22 and pine bark on the microbial diversity and denitrification activity in the treatment of high
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24 strength nitrified landfill leachates (nitrate concentrations ranging between 500 up to 2000
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26 mg l⁻¹). The growth of the microbial community was followed using a spread plate
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28 enumeration technique; the colonization of the substrates was assessed through
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30 Environmental Scanning Electronic Microscopy (ESEM), and insight into the composition
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32 of the bacterial community was obtained by phylogenetic analysis.
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39 **2. Materials and methods**

40 **2.1. Leachate selection**

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42 To avoid analytical interferences, treated leachate from an SBR was simulated in the
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44 laboratory with a synthetic solution of potassium nitrate and distilled water with a
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46 concentration of 500 mg l⁻¹ of NO₃⁻.
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52 **2.2. Carbon sources selection**

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54 Commercial (CGR) and domestic (DGR) garden refuse and pine bark (PB) were collected
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56 at the Mariannhill landfill site (Durban, South Africa). The garden refuse was composted
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for 10 weeks in pilot-scale forced aerated vessels at the University of KwaZulu-Natal (Durban, RSA). The properties of the solid substrates were characterized according to standard analytical methods as published by the American Public Health Association [18]: moisture content, total solids, volatile solids, C/N, Dynamic Respiration Index at 7 days (DRI₇), determined with an OxiTop® respirometric system. Eluate tests were conducted to assess amount and nature of the compounds leached-out from the substrates in distilled water during 24 hours, using a 10/1 Liquid to Solid ratio (L/S) [11]. The following parameters were measured: total solids, volatile solids, pH, conductivity (ρ), COD, BOD₅, NH₃, NO_x and C/N ratio. All analyses were carried out in triplicate.

2.3. Batch tests

Batch experiments were designed to study the denitrification patterns of the synthetic leachate using the three substrates as carbon sources. Duplicate tests were conducted in 1.5 L anaerobic bottles equipped with two airtight silicone septa that allow for continuous sampling avoiding air ingress. Each substrate (S) was mixed with the synthetic leachate (L) at L/S=10/1 (Table 1). A control test with distilled water was also carried out for each substrate. Optimal environmental conditions and full liquid to solid transfer were obtained by performing the experiments at a controlled temperature of 25 °C and by shaking at 150 rpm. The size of the pine bark chips had to be reduced to 2-3 cm and the batches were flushed with N₂ to set anaerobic conditions.

For nitrate and pH testing, 2 mL samples were collected with a precision syringe connected to a 0.45 μ m filter after 5, 10, 15, 30 and 60 minutes and then every hour during the first

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day. Afterwards, samples were collected four times a day. Nitrate and nitrite concentrations were analyzed using Nitrate Test Sticks type Merckoquant (MERCK). This method was selected to avoid large variations of the L/S ratio with time maintaining a reasonable accuracy (error within 10-15%). 1.5 mL samples were taken three times a day with a sterile syringe for microbiological analyses from Batch 1 for each substrate (Figure 1, 2, and 3). The experiment was stopped when total denitrification was achieved, except for the pine bark for which the final concentration never fell within the discharge limits during the experimental time. The output COD, ammonia and pH were then analyzed on the filtered eluates.

2.4. Batch inoculation

In order to investigate the effect of inoculation on the reaction rate and the acclimatization time, 5 ml of solution of the first CGR test, were used to inoculate a second CGR batch prepared in the same conditions.

2.5. Semi-quantitative analysis of the bacterial community

The effect of the substrates on the growth of the bacterial populations was assessed during the batch tests. The 9215-C spread plate method [18] was applied to enumerate the aerobic cultivable microflora. A laminar flow cabinet was used to work in a sterile atmosphere. Samples were diluted in sodium chloride solution at 9 g l⁻¹ and 100 µl of each dilution (10⁻³ to 10⁻⁷) were spread on 90 mm agar plates using the Luria-Bertani Broth. The glass rods were replaced by 4 mm glass beads spread on the plate. Plates were incubated at room temperature (25 °C) in the dark and enumeration was carried out visually after 3 days [19].

2.6. Microscopic analysis of the bacterial community

Colonization of the different solid substrates was assessed using an Environmental Scanning Electronic Microscope (ESEM Philips, FEI XL 30). Samples were fixed in 3% (v/v) glutaraldehyde, washed twice in 0.05 M cacodylate buffer (pH 7.1) for 10 min and dehydrated in an alcohol series (10 min each in 30%, 50%, 70%, 80%, 90%, and 3×10 min in 100%) in a fume cupboard. The specimens were then transferred into critical point drier baskets under 100% alcohol and dried in a pre-cooled Hitachi HCP-2 critical point drier. After gold palladium sputter coating (Polaron Equipment Limited SEM, coating unit E5100), the samples were examined in the ESEM at 10 keV.

2.7. Genetic analysis of the bacterial community

DNA extraction from the PB (at 2.5 h, 96 h and 263 h) and the CGR (at 3 h, 74 h and 162 h) liquid samples was carried out using the Zymo Research Fungal/Bacterial DNA extraction kit as described by Lejon et al. [20]. Purified DNA was suspended in 50 µL of sterile water and examined by agarose gel electrophoresis. All extracted genomic DNA samples were stored at –20 °C until further processing. The 16S rRNA gene was amplified by PCR using universal bacterial 16S primers 27-F and 1492-R [21]. PCR amplification was performed using Lucigen EconoTaq plus Green master mix. PCR products (approximately 1400 bp) were analyzed on a 1% agarose gel and cleaned with PCR purification kit (Qiagen). PCR products were then cloned using the CloneJet kit (Fermentas) according to the manufacture's specifications. The screening of inserts from the transformants was performed by direct PCR amplification from colonies using primers for the pJET1.2F and pJET1.2R sites on the plasmid. Amplified inserts were identified on

gel electrophoresis and cleaned by using the ZR-DNA Sequencing Clean-up kit™ (Zymo Research Corp). DNA sequences were determined by using an ABI 3130XL genetic analyzer and the BigDye terminator v3.1 cycle sequencing kit (PE Applied Biosystems). Sequences were compared to the GenBank nucleotide database library by BLAST on-line searches [22].

3. Results and discussion

3.1. Substrates characterization

Characterization of the solid matter showed that different origins and composition of the domestic garden refuse in relation to the commercial sample are evident (Table 2). Primarily large palm leaves, grass and twigs constituted the former, while the latter contained largely woody waste, tree bark and branches that made it more similar to the pine bark. These differences in composition, associated with the substrates' origins and collection methods, reflect also on the amount and nature of the available carbon for denitrification which was two times higher in CGR than in DGR (Table 2). The high C/N ratio for the pine bark fell within the expected range as in literature, while the low value for the compost suggested an IV and V degree of maturity for the CGR and DGR, respectively (DIN 4187), with levels slightly higher than the optimum range of 13-16 for stabilised garden refuse compost [11]. Overall, CGR and PB displayed similar characteristics with respect to their composition, origin and C/N before composting suggesting a similarity in the way carbon is released during denitrification. It is also worthy to note that high amounts of nitrogen, COD and TS are released from the DGR after 24 hours contact time with water (eluate tests) as well as during the batch tests (Table 3) through mechanisms of bio-leaching

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5 as observed also by other authors [6; 13; 23]. During this initial period ammonia is
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7 promptly converted into nitrites by nitrifiers as oxygen is still trapped in the water, while
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9 denitrification is limited by the availability of electron donors with a consequent increase in
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11 nitrate concentrations [6].
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15 16 17 **3.2. Batch tests**

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19 Although no significant differences were observed for the DGR, CGR and PB Batch tests
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21 in term of nitrate removal, each substrate showed a distinct biotransformation rate. In the
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23 test with the CGR, complete removal of nitrates in solution was achieved within 8 days
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25 (Figure 1). The DGR tests showed a large initial release of nitrate (500 mg l^{-1}) in solution
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27 by the substrate, independently of the input nitrate concentration (Figure 1). However, the
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29 nitrate consumption rate remained close to that of the CGR tests and the complete
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31 denitrification was achieved within 8 days. The onset of denitrification was generally
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33 slower in the tests with PB and complete nitrate removal was not achieved, as the final
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35 concentration plateaued around 150 mg l^{-1} after 11 days (data not shown). This finding
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37 suggests the occurrence of a strong inhibitory effect on the active denitrifier population.
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39 Further this could be explained by the low pH observed during the batch tests with PB
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41 (Table 4), as suggested by other studies [6, 13, 23]. Although a neutral pH in the batch
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43 tests with compost could suggest a more favourable condition for microbial activity, the
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45 high release of COD and nitrate in the DGR is of concern and would require further
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47 investigation (Table 4).
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59 **3.3. Initial inoculation effect**

No direct effects were observed on nitrate removal in the inoculated batch test (data not shown). In a denitrification study using newspapers as a carbon source, Volokita et al. [12] found that an initial inoculation with a solid matrix was far more efficient than with a liquid inoculum. On the contrary, Ovez et al. [9] reported an inhibitory effect when inoculating their batches with bacteria from previous experiments. These contrasting effects might be explained by the extreme complexity of the microbial community established during the denitrification process, which is strongly dependent on the nature of the substrate and the experimental conditions. In general, inoculation using a solid substrate containing a well-established microflora should be preferred to an inoculum derived from the liquid phase.

3.4. Effect of the solid substrates on the size of the aerobic bacterial community

The number of colony forming units (CFU) for both the CGR and DGR (Figure 2) was estimated to be 5.10^7 CFU ml⁻¹ at the beginning of the experiment and decreased by five orders of magnitude during the first two days. The viable bacterial community present in the PB test at the beginning of the experiment was accounted to 3.10^8 CFU ml⁻¹ which is ten times higher than in the compost tests (Figure 2). A logarithmic decrease ($R^2=0.94$) was observed during the first 7 days, leading to a constant bacteria concentration of 1.10^7 CFU ml⁻¹ until the test was stopped. Assuming CFU were mainly using carbon and nitrate for their development, it should be possible to establish a relationship between CFU numbers and denitrification rate. The correlation between these two parameters for the liquid phase of the PB batch tests was good ($r^2 > 0.80$) and confirmed that carbon and nitrate depletions were mainly related to the microbial activity. Whilst this finding is in agreement with previous studies [6, 7, 8], it should be interpreted with much care. Indeed, the enumeration

of the bacteria in the liquid phase does not account for those proliferating on the surface of the substrates (biofilms), and as such, it may not constitute a reliable indicator [7].

3.5. Effect of the solid substrates on the bacterial community

Phylogenetic analysis was performed on 16S ribosomal DNA for each treatment in order to obtain further insight into the bacterial community structure and dynamics (Table 2). Even if the same tendencies were observed for the three treatments (dominance of *Gamma-proteobacteria*, *Firmicutes* and *Bacteroidetes* in all libraries) differences were observed between composts and PB applications. During the acclimatization period, the bacterial community observed in the CGR and DGR tests was essentially composed of *Gammaproteobacteria* commonly found in natural environments, e.g. *Pseudomonas putida*, *Pseudoxanthomonas*, *Rheinheimera sp.* [24]. In contrast, the PB test was dominated by Enterobacteria including *Rahnella*, *Pantoea*, *Kluyvera* and *Enterobacter* which are typical of pine bark [25]. The population of *Rahnella sp.* largely dominated during the experiment while *Pantoea agglomerans* disappeared halfway through the experiment as being outcompeted by *Lactobacillus* and *Erwinia sp.* which both are known to be unable to reduce nitrate [26]. *Enterobacter sp.* and *Pantoea agglomerans* are potential human pathogens [24] and as such could prevent the applicability of the pine bark in full-scale operations. .

Bacteria capable of reducing nitrate into ammonia such as *Acinetobacter sp.* for *Gammaproteobacteria* [27] and *Clostridium sp.* for *Firmicutes* [28] as well as bacteria capable of dissimilatory nitrate reduction such as *Rhizobium sp.* and *Thermomonas sp.* [29]) were identified after 74 hours treatment in the CGR eluates. In contrast, dominance of

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Thermoactinomyces in the DGR eluate after 74 hours suggests that these bacteria can first produce nitrous acid from nitrate followed by the generation of ammonium as they have both nitrate-reducing and ammonium-forming ability [30]. Over time, the bacterial community in the CGR eluate evolved towards a consortium of denitrifiers mainly composed of *Rheinheimera sp.*, *Phaeospirillum sp.* and *Flavobacterium sp.* [23, 30, 31]. *Phaeospirillum sp.* has been described as being able to use ammonia as a nitrogen source [32]. This suggests that it could counterbalance the presence of the ammonia-producing bacteria present in the second step of the experiment. This hypothesis is further supported by the low concentration of ammonia found at the end of the experiment ($\text{NH}_3\text{-N}=3 \text{ mgL}^{-1}$)

3.6. Bacterial colonization of the solid substrates

The interpretation of ESEM micrographs could be challenging as the preparation of the samples may significantly change the matrix structure through shrinking and deformation [33]. To overcome this limitation, solid substrates before and after treatment were compared. Before treatment, cocci and fungal spores were the two most abundant organisms colonizing the surfaces of CGR while numerous cocci and rod-shaped bacteria were observed on the surfaces of DGR (Figure 3 and 4). After treatment, rod-shaped bacteria dominantly colonized CGR surfaces (Figure 5) whilst no changes were observed in DGR tests (data not shown). This finding suggests that the composted domestic garden refuse (DGR) offers a favourable surface for the rapid development of a biofilm of denitrifiers and that NO_3^- exerts a selective pressure on promoting the growth of rod-shaped bacteria leading to the formation of a superficial biofilm.

Numerous cocci were visible on the surface of the PB before incubation (Figure 6). After treatment, very few bacterial cells were observed in the control and nitrate-rich tests (data not shown) due to possible inhibitory effects or desorption of most of the bacteria from the surface of the pine bark into the liquid phase. Previous studies demonstrated that pine barks could release large amounts of phenolic compounds and hydroxylated benzene rings, which both can inhibit the activity of various bacterial enzymes [32, 33]. Added to this, a constantly low pH during the process did not contribute in creating favourable conditions to bacterial growth.

4. Conclusion

The composts (CGR and DGR) proved to be efficient substrates for denitrification, promoting the sustained development of a complex biofilm as a niche for the denitrifying communities. The phylogenetic analysis carried out on CGR and DGR samples showed that the bacterial community evolved from a diverse community towards a limited consortium of active denitrifiers. Pine bark was found to be far less efficient in promoting favourable conditions for microbial growth because of the combined effect of a low pH and the release of potentially inhibitory compounds leading to the irreversible release of biofilm forming cells into the leachate. Furthermore, potential pathogens have been detected in association with the pine bark, rendering unsuitable its use as a carbon source for the treatment of nitrate-rich leachates at a large scale. Overall, this study contributes in pointing out the different behaviour displayed by the microorganisms from different substrates in the solid and liquid phases and highlights the important role of biofilms in the denitrification process and their sensitivity to prevailing environmental conditions.

Acknowledgements: This study was conducted under the aegis of the NRF (SA) and CNRS (France) Scientific Collaboration Agreement. The authors would like to thank Durban Solid Waste for financial support; the Microbiology and Plant Pathology Departments of the University of KwaZulu-Natal (Pietermaritzburg) for sharing their expertise; Sheila Arnold, Bjorn Plug, Dr Daniel Teclu (UKZN) and Inquaba Bio-Tech Laboratories for assisting with the analytical work. We also thank Ewan Vince of the University Joseph Fourier (Grenoble) for his technical assistance and the platform MOME from the pole Envirhonalp.

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Table 1 Batch experiment set-up

	Mass of substrate (g)	Volume of the solution (ml)	Concentration of the solution (g.l ⁻¹ of KNO ₃ ⁻)
CGR	313	787	1.4
DGR	295	805	1.0
PB	196	905	0.9

Commercial (CGR) and (DGR) domestic garden refuse; Pine bark (PB)

Table 2 Solid matter characterisation for each substrate

	Moisture Content (%)	Total Solids (%)	Volatile Solids (%)	RI ₇ (mgO ₂ /g DM)	C/N (before composting)	C/N
CGR	67 ± 1	33 ± 1	47 ± 2	8.5 ± 0.6	40	24
DGR	66 ± 6	34 ± 6	62 ± 12	14.1 ± 0.4	22	13
PB	49 ± 3	51 ± 3	97.1 ± 0.1	17.8 ± 0.4	n.a.	62

Table 3 Physicochemical characteristics of the eluates after 24 hrs

	Total Solids (g l ⁻¹)	Volatile Solids (g l ⁻¹)	pH at 20°C	ρ (mS/cm) at 20°C	COD (mg l ⁻¹)	BOD ₅ (mg l ⁻¹)	NH ₃ -N (mg l ⁻¹)	NO _x -N (mg l ⁻¹)	C/N
CGR	2.4 ± 0.1	1.6 ± 0.1	6.9	0.81	2800 ±400	155	9.8 ± 1.2	0.19 ± 0.05	1.8
DGR	17 ±3	12 ±0.2	7.5	5.1	17600 ±1300	350	82.0 ± 0.4	8 ± 2	8.3
PB	3.6 ± 0.01	3.3 ±0.3	4.2	0.85	4500 ±450	297	8.5 ± 0.1	0.03 ± 0.01	3.6

Table 4 Evolution of COD, pH and NH₃ in the liquid phase during the batch tests

	COD (mg l ⁻¹)	pH at 20°C	NH ₃ -N (mg l ⁻¹)
CGR 24h	2800 ± 400	6.9	9.8 ± 1.2
CGR final	3200 ± 100	7.5	3.0 ± 0.1
DGR 24h	17600 ± 1300	7.5	82.0 ± 0.4
DGR final	17800 ± 1100	7.6	87.2 ± 0.4
PB 24h	4410 ± 20	4.2	8.5 ± 0.1
PB final	14200 ± 1200	4.3	22.5 ± 1.0

Table 5: Summary of bacterial sequence identification (expressed as %) according to the closest matches to sequences in the Genbank database found by BLAST

	PB eluate			CGR eluate			DGR eluate		
Phylogenetic group / genus level	2.5 h	96 h	263 h	3 hr	74 h	162 h	3 hr	74 hr	162 hr
α-proteobacteria									
<u>Phaeospirillum</u>						26	-	-	-
<u>Rhizobium</u>				-	20	8	-	-	-
Alpha proteobacterium INAWF007							-	7	3
Aquicella siphonis				-	-	-	21	12	8
γ - proteobacteria									
Pseudoxanthomonas	-	-	-	30	4	-	4	-	-
<u>Rheinheimera</u>	-	-	-	25	9	14	11	-	-
<u>Acinetobacter</u>	-	-	-	-	12	2	4	-	-
<u>Pseudomonas</u>	-	-	-	-	-	17	12	8	5
Thermomonas	-	-	-	-	20	5	-	-	-
<u>Rahnella</u> *	24	22	34	-	-	-	-	-	-
Pantoea*	12	-	-	-	-	-	-	-	-
Kluyvera*	6	6	4	-	-	-	-	-	-
Enterobacter*	14	11	8	-	-	-	-	-	-
Uncultured gamma proteobacterium clone 16S5	11	-	-	-	-	-	-	-	-
Erwinia*	-	22	10	-	-	-	-	-	-
Firmicutes									
Uncultivated clostridium sp clone 3.28	-	-	-	8	7	-	-	-	-
Geobacillus				23	3	-	-	-	-
Bacillus	-	-	-	-	-	-	13	29	40
Thermoactinomyces	-	-	-	-	-	-	2	24	27
Lactobacillus	-	33	29	-	-	-	-	-	-
Bacteroidetes									

<u>Flavobacterium</u>	-	-	-	-	-	17	-	-	-
<u>Pedobacter</u>	-	-	-	-	18	3	-	-	-
unknown	33	6	15	14	7	8	33	20	17

Phylogenetic grouping based on the highest identity score obtained after submitting the sequence to BLAST (sequence identity with > 97% homology). Data are expressed as % of 16S rRNA clones.

“-“ : not detected; * Enterobacteria

Figure(s)

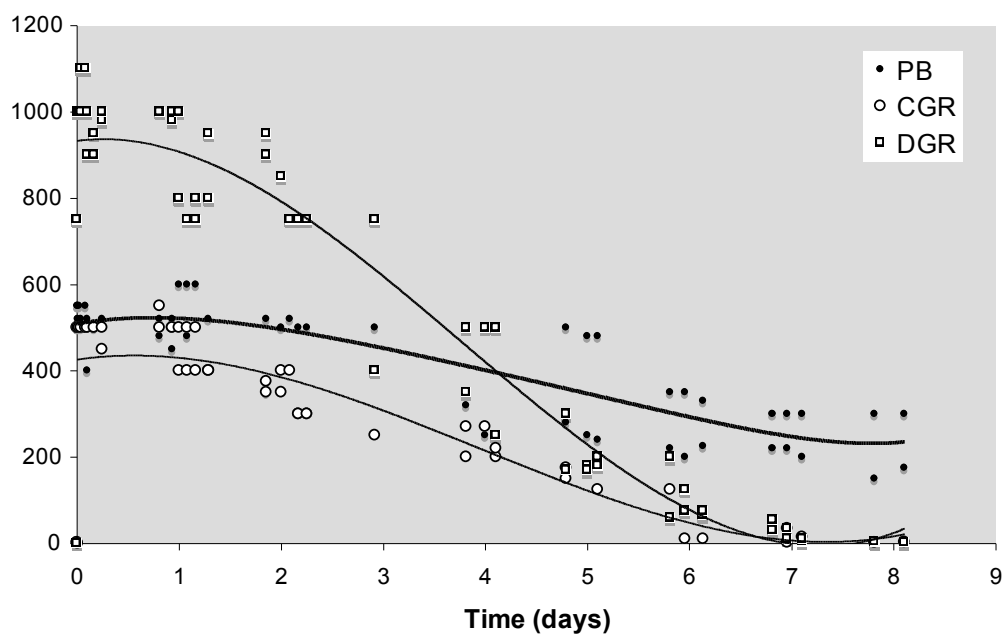


Figure 1: Influence of Pine Bark (PB), Commercial Garden Refuse (CGR) and Domestic Garden Refuse (DGR) amendment on the nitrate removal in batch tests.

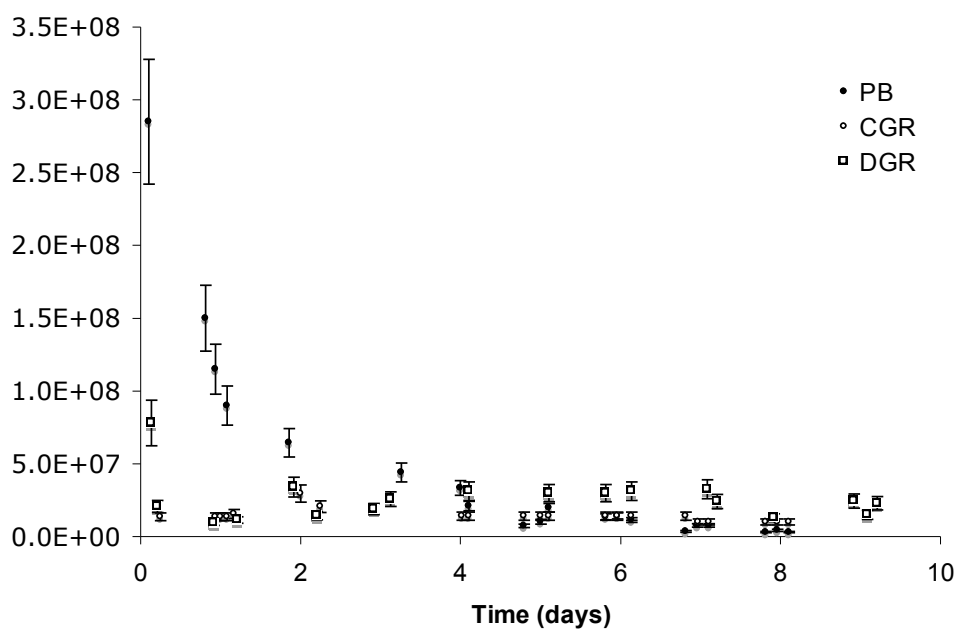


Figure 2: Change in the abundance of the microbial population according to the carbon sources used in batch tests.

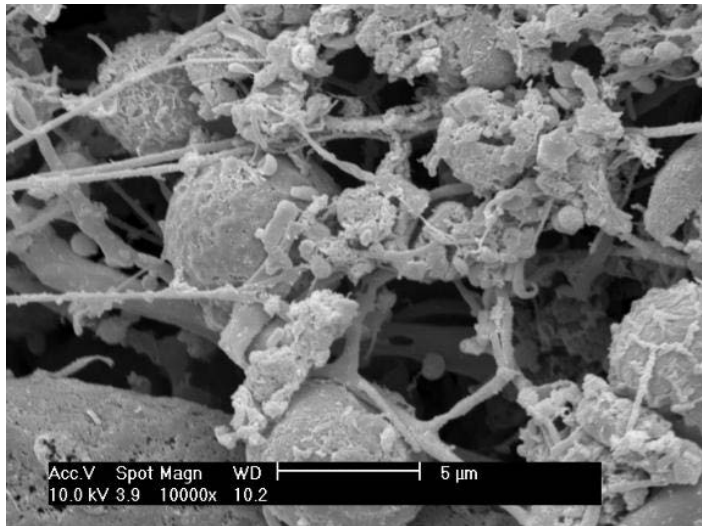


Figure 3: ESEM micrograph of the surface of CGR before incubation



Figure 4: ESEM micrograph of the surface of DGR before incubation

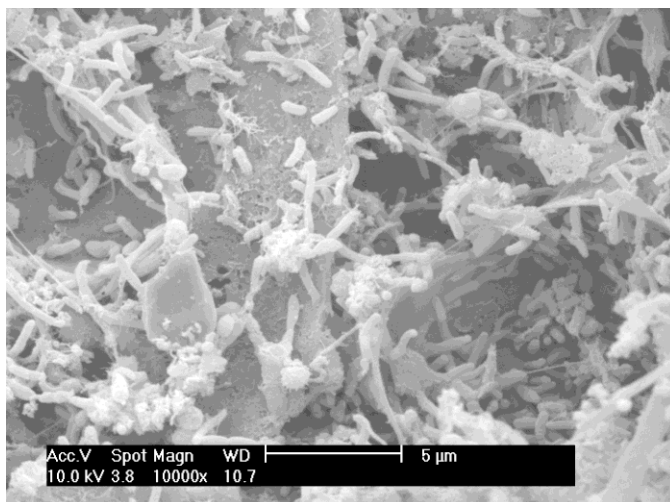


Figure 5: ESEM micrograph of the surface of CGR 500 after 8 days

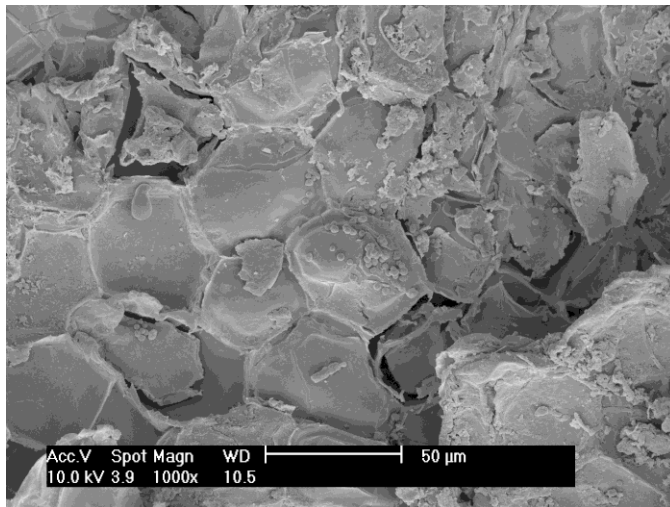


Figure 6: ESEM micrograph (magn. x 10000) of the surface of pine bark (PB) before incubation